Biogeographical and phylogenetic origins of African fig species (*Ficus* section *Galoglychia*)

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Abstract

Ficus section *Galoglychia* (subgenus *Urostigma*; Moraceae) includes 72 species restricted to the African floristic region (a few extending to the Arabian Peninsula and Socotra). We present the first molecular phylogenetic analysis of the section including 56 ingroup (representing 44 species) and three outgroup taxa, to investigate its monophyly, classification and evolution. We used sequence data from the nuclear ribosomal internal and external transcribed spacers (ITS and ETS). Our results suggest that section *Galoglychia* is paraphyletic to the neotropical section *Americana*, although this is not supported by bootstrap analysis and only weakly supported by Bayesian posterior probabilities. Maximum parsimony analysis conflict with maximum likelihood and Bayesian analyses with respect to the closest relatives of section *Americana* in Africa. The subsections of section *Galoglychia* proposed by Berg [Berg, C.C., 1986. Subdivision of *Ficus* subg. Urostigma sect. Galoglychia (Moraceae). Proc. Kon. Ned. Akad. Wetensch., Ser. C, 89, 121–127] are generally supported. We find two major clades of section *Galoglychia* within Africa possibly corresponding to two main centres of diversity. One clade comprises members of subsections *Platyphyllae* and *Chlamydodorae*, which are more concentrated in Eastern Africa, and extend to Madagascar and neighbouring archipelagos (Comores, Mascarenes, Aldabra Islands and Seychelles). The other main clade includes members of subsections *Caulocarpae*, *Cyathistipulae*, *Crassicostae* and *Galoglychia*, which are concentrated in West and Central Africa.
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1. Introduction

Ficus (Moraceae) constitute one of the largest genera of angiosperms (Frodin, 2004), with almost 800 species of terrestrial trees, shrubs, hemi-epiphytes, climbers and creepers occurring in the tropics and subtropics worldwide. All members of the genus share the distinctive fig inflorescence (syconium), which is the site of an extreme mutualism with pollinating fig wasps of the family Agaonidae (Cook and Rasplus, 2003). *Ficus* are important genetic resources with high economic and nutritional value. They are also an important part of the biodiversity in the rainforest ecosys-

tem by setting fruit throughout the year and providing an important source of food for fruit-eating animals in the tropics.

The African floristic region contains about 105 species of *Ficus* of which 72 constitute *Ficus* section *Galoglychia* (Gasp.) Endl. (Berg and Wiebes, 1992). The section is nearly confined to the region (a few species extend to the southern part of the Arabian peninsula and Socotra) and includes nine species in the Madagascar region, three of them also occurring in mainland Africa (Berg, 1986; Berg and Wiebes, 1992). Section *Galoglychia* belongs to the monoecious and worldwide subgenus *Urostigma* and is closely related to the neotropical section *Americana* (Jousselin et al., 2003; Rønsted et al., 2005).

Members of section *Galoglychia* are characterised by a slit-shaped (or occasionally tri-radiate) ostiole with all ostiolar bracts descending, two basal bracts and di-thecal

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anthers (Berg, 1986). The section is more diverse (in vegetative parts, dimensions and position of the syconia) than the other sections of subgenus *Urostigma* (Berg, 1986). Most species are evergreen hemi-epiphytes that establish themselves in crevices within the canopy and send down a root along the trunk of a host tree. With time the root-system of these *Ficus* expands around the trunk of the host by anastomosis and these potential 'strangler' species may eventually kill the host. Hemi-epiphytes can also settle on rocks and some species (e.g. *F. abutilifolia* and *F. tettensis*) are hemi-epilithic and may split their substrate. Other species (e.g. *F. platyphylla*) are primarily terrestrial and adapted to dry habitats of savannah woodlands, and a few species (e.g. *F. lingua* and *F. ottonifolia*) may be lianas. Leaf size may vary among species from a few cm to half a meter long, and are usually entire and coriaceous. Syconia are mostly borne in pairs in the leaf axils or, in some species, on short spurs on the trunk and older branches. The syconia can be from half a centimetre to about 10 cm in diameter and contain from about ten to thousands of minute flowers depending on the species and the size of the syconia. Berg and Wiebes (1992) provide a detailed discussion of the structure of the syconia and flowers. The diversity of section *Galoglychia* is also reflected in the number of genera of Agaonidae involved in their pollination (Table 1; Berg and Wiebes, 1992).

The African species of *Ficus* were first revised by Mildbraed and Burret (1911), who included 73 species from subgenus *Urostigma* Miq. in a newly recognized subgenus, *Bibracteatae* (= section *Galoglychia* (Gasp.) Endl.), a name that refers to the presence of two bracts at the base of the receptacle common in the group, although this name is misleading because this feature turned out not to be a good distinguishing character (Hutchinson, 1915). Mildbraed and Burret divided their subgenus into seven sections based on morphological and ecological affinities: i.e. *Platyphyllae*, *Caulocarpae*, *Fasciculatae*, *Elegantes*, *Crassicostae*, *Cyathistipulae*, and *Chlamydodorae*.

The description of several new African species of *Ficus* since Mildbraed and Burret's (1911) treatment of the group, encouraged Hutchinson (1915) to propose a new subdivision of the subgenus for his treatment for the Flora of Tropical Africa. The species were divided into two series, emphasising a few key-characters: plants bearing syconia in fascicles (series *Fasciculatae*, including species from sections *Caulocarpae*, *Fasciculatae*, and *Elegantes*), versus singly or in axillary pairs (series *Axillares*, which was divided into subseries *Stipulares* (= section *Cyathistipulae*) and subseries *Cauducae* (= sections *Platyphyllae*, *Crassicostae* and *Chlamydodorae*)).

Lebrun (1934) resurrected most of Mildbraed and Burret's sections noting that many of them were probably not "natural". Section *Elegantes* was included in *Caulocarpae*, and section *Crassicostae* in *Chlamydodorae*. Two of Hutchinson's (1915) informal groups from series *Cauducae* were proposed as new sections: *Furcatae*, including species with obtriangular leaves and the midrib bifurcating towards the apex, and *Chlamydocarpae*, including species with large basal bracts covering the young receptacles.

Berg (1986) based his subdivision on that of Mildbraed and Burret (1911), but ranked *Galoglychia* as a section with six subsections (see also Table 1): *Caulocarpae* (11 spp.), *Chlamydodorae* (13 spp.), *Crassicostae* (8 spp.), *Cyathistipulae* (19 spp.), *Galoglychia* (3 spp.), and *Platyphyllae* (18 spp.). The circumscriptions of subsections *Platyphyllae* and *Chlamydodorae* were almost identical to Mildbraed and Burret's (1911) corresponding sections, while sections *Crassicostae* and *Cyathistipulae* were largely recircumscribed and some of the species previously placed here, were included in the new subsection *Galoglychia*. Mildbraed and Burret's (1911) sections *Fasciculatae* and *Elegantes* were included in an expanded subsection *Caulocarpae*. Berg and Wiebes (1992) considered the taxonomy of African *Ficus* to be sorted out satisfactorily although there remain doubts about the correct position of some of the species and the delimitation of other species (e.g. *F. thonningii* complex).

The first molecular phylogenetic analysis of *Ficus* (Herre et al., 1996) included only 15 species of *Ficus*. Their study was based on plastid *rbcL* and tRNA sequences, which provided only low resolution. The only representative of section *Galoglychia*, *F. natalensis* Hochst., was found to be sister to *F. macrophylla* Desf. ex Pers. (section *Malvanthera*) and more distantly related to section *Americana*. A study by Weiblen (2000) based on nuclear ribosomal ITS sequences included 46 species of *Ficus*, but focused on dioecious *Ficus* and did not include representatives of section *Galoglychia*. Jousselin et al. (2003) used a combination of ITS and ETS data of 41 species of *Ficus* and included representatives of most sections of *Ficus*. In their study, sections *Galoglychia* (*F. lutea* Vahl, *F. glumosa* Delile, and *F. cyathistipuloides* De Wild.) and *Americana* (*F. nymphaeifolia* Mill.

Table 1
Subdivision of *Ficus* section *Galoglychia* (Berg, 1986; Berg and Wiebes, 1992)

Subsection	No. of species	Distribution	Habitat	Genera of pollinating wasps
Caulocarpae	11	Africa, 1 sp. extends to Madagascar	Wet to dry evergreen forest	*Agaon*, *Courtella*
Chlamydodorae	13	More concentrated in Eastern Africa, 2 spp. on Madagascar and neighbouring islands	Rainforest or savannah woodland	*Alfonsiella*, *Elisabethiella*
Crassicostae	8	West and Central Africa, 1 sp. in East Africa	Rainforest	*Elisabethiella*, *Paragaon*
Cyathistipulae	19	West and Central Africa, 1 sp. in East Africa	Rainforest	*Agaon*
Galoglychia	3	West and Central Africa	Evergreen forest	*Allotriozoon*
Platyphyllae	18	Concentration in Eastern Africa, 5 spp. in Madagascar and neighbouring islands	Mainly savannah woodland, adapted to dry habitats, some rocksplitters	*Alfonsiella*, *Elisabethiella*, *Nigriella*

and *F. schumacheri* (Liebm). Grieseb.) were strongly supported as sister groups (99% Bootstrap support, BS). However, there are many potential problems associated with using only exemplar taxa to represent large groups. Exemplar taxa may not adequately represent the variation present in the group they represent, and inadequate sampling may also influence phylogenetic accuracy (e.g. see Graybeal, 1998).

In a more comprehensive study by Rønsted et al. (2005), also based on ITS and ETS, including 146 taxa of *Ficus*, sections *Galoglychia* (25 taxa sampled) and *Americana* (24 taxa sampled) were also found to be sister clades, although the former received less than 50% BS and in 47% of the most parsimonious trees, it was found to be paraphyletic with respect to section *Americana*. The purpose of the present study is to (1) examine the circumscription of section *Galoglychia* with respect to section *Americana* using sequences of the nuclear ribosomal internal and external transcribed spacers (ITS and ETS), (2) evaluate relationships within section *Galoglychia*, and (3) use the results of the phylogenetic analyses to evaluate the biogeography of the section.

2. Materials and methods

2.1. Materials

Total genomic DNA was extracted from 35 taxa of *Ficus* using CTAB (Doyle and Doyle, 1987). In addition 31 ITS sequences and 32 ETS sequences were retrieved from GenBank/EBI (following papers originally published by Weiblen, 2000; Jousselin et al., 2003; Rønsted et al., 2005), resulting in a total sample of 56 taxa (representing 44 species) of *Ficus* section *Galoglychia*, eight of section *Americana* and three representing the remaining sections of subgenus *Urostigma*. Included material, voucher information, origin and GenBank/EBI accession numbers (DQ455636–DQ455688) are listed in Table 2. Our sampling encompass all six subsections of *Ficus* section *Galoglychia* sensu Berg (1986).

2.2. DNA extraction, amplification and sequencing

DNA extractions were performed using 0.2–0.3 g silica-dried leaves and a modified version of the $2 \times$ CTAB method of Doyle and Doyle (1987). Before precipitation, an aliquot was purified using Qiagen PCR purification kit (Qiagen, Santa Clarita, CA, USA) following the manufacturer's protocols. The remainder of the DNA was purified using a caesium chloride/ethidium bromide gradient (1.55 g/ ml density) followed by a dialysis and was deposited in the DNA Bank at the Royal Botanic Gardens, Kew (www.kew.org).

The internal and external transcribed spacers, ITS and ETS (Baldwin et al., 1995; Baldwin and Markos, 1998), were amplified using primers 17SE and 26SE (Sun et al., 1994) and Hel1 and 18S ETS (Baldwin and Markos, 1998),

respectively. All PCR reactions were 50 µl reactions and DMSO (2%; dimethylsulfoxide) and BSA (bovine serum albumine) were added to all reactions. PCR mastermix including 1.5 mM $MgCl_2$ (Bioline, London, UK) was used to amplify ITS. ETS reactions was prepared using Biotaq DNA polymerase (Bioline); $10\times$ KCl buffer (Bioline) or Opti-Prime master mix $50\times$ buffer and Opti-Prime $10\times$ buffer #10 containing KCl (Stratagene, Cambridge, UK); dNTP's, (Promega, Crawley, West Sussex, UK); $MgCl_2$; BSA, and 2% of DMSO, per reaction. Amplification of ITS consisted of 2 min at 94 °C followed by 28 cycles of: 1 min denaturation (94 °C), 1 min annealing (50 °C) and 3 min extension (72 °C). After the last cycle, the temperature was kept at 72 °C for a final 7 min extension and then lowered to 4 °C. Amplification of the ETS region consisted of 4 min at 94 °C followed by 30 cycles of 1 min denaturation (94 °C), 1 min annealing (50 °C) and 1 min extension (72 °C). After the last cycle, the temperature was kept at 72 °C for a final 2 min extension. All PCR and cycle sequencing reactions were run on a Perkin–Elmer GenAMP® model 9600 or 9700. Amplified products were purified with the Qiagen PCR purification kit (Qiagen) following the manufacturer's protocols.

Cycle sequencing reactions were carried out using the BigDye™ Terminator Mix (Applied Biosystems, Foster City, CA). The sequencing protocol consisted of 26 cycles of 10 s denaturation (96 °C), 5 s annealing (50 °C) and 4 min elongation (60 °C). Products were run on an ABI 3100 Genetic Analyzer according to the manufacturer's protocols (Applied Biosystems). Both strands were sequenced for each region for all but one taxon (due to sequencing failures for the ETS region, *F. cyathistipuloides* was only sequenced with the primer 18S). Seven taxa were only sequenced for ITS because ETS could not be amplified (*F. amadiensis*, *F. conraui*, *F. dryepondtiana*, *F. faulkneriana*, *F. nigropunctata*, *F. oreodryadum*, and *F. reflexa*). In addition, duplicates of seven taxa were sequenced for ITS to evaluate potential problems of infraspecific variation (*F. dryepondtiana*, *F. fischeri*, *F. oreodryadum*, *F. ovata*, *F. platyphylla*, *F. polita*, and *F. preussii*). For this study, 52 sequences were generated and submitted to GenBank; accession numbers are listed in Table 2.

2.3. Alignment and phylogenetic analyses

Sequences were edited and assembled using Sequencer 4.1.2™ software (Gene Codes Corp., Ann Arbor, MI, USA), and following the guidelines of Kelchner (2000) all sequences were easily aligned by eye in PAUP v. 4.0b5 for Macintosh (Swofford, 2002).

We assembled a matrix including ITS and ETS sequences of 39 taxa of section *Galoglychia*, eight taxa of section *Americana*, and an outgroup including one taxon each from the three remaining sections of subgenus *Urostigma*, namely sections *Conosycea* (*F. drupacea*), *Malvanthera* (*F. rubiginosa*), and *Urostigma* (*F. superba*) based on previous studies (Jousselin et al., 2003; Rønsted et al., 2005).

Table 2
Taxa from the African *Ficus* section *Galoglychia* and outgroups included in the study

Taxon	Distribution[a]	Voucher	Origin	ITS	ETS
Subsection *Caulocarpae*					
Ficus bizanae Hutch & Burt-Davy	RSA	Forest 334 (NBG)	LC (NBG), 186-82	DQ455636	DQ455670
Ficus bubu Warb.	W Ang, Mal, Moz, Zim, Swa, RSA, ext. to Ken, Uga, Ivo and Sen	Forest 339 (NBG)	LC (NBG), 430-84	DQ455637	DQ455671
Ficus dryepondtiana L	Zai, CAR, Con, Cam, Gab	Rønsted 209 (K)	Cameroon	DQ455638	—
Ficus dryepondtiana L		FB/S2800 (BR)	LC (BR)	DQ455639	—
Ficus ottonifolia (Miq.) Miq. ssp. *macrosyce* Berg	N Ang, N Zam, Zai, Gab, Uga, Mal, Tan, Ken, ext.to Sen, Fer			AY730109[b]	AY730198[b]
Ficus ovata Vahl.	N Ang, N Zam, Mal, Moz, ext. to Eth and Sen	Rønsted 205 (K)	Cameroon	DQ455640	—
Ficus ovata Vahl.		Rønsted 132 (K)	LC (BG), 94.425	—	DQ455672
Ficus ovata Vahl.		Rønsted 235 (K)	Cameroon	DQ455641	—
Ficus polita Vahl. ssp. *polita*	Sen to Uga and N Ang, Ken, Tan, Moz, RSA, Mad	Maurin et al. 88 (K)	Madagascar	DQ455642	DQ455673
Ficus polita Vahl. ssp. *polita*		Rønsted 150 (NR)	LC (BG), 88.269	DQ455643	—
Ficus sansibarica Warb.	Zam, N Ang, Zai, Mal, NE RSA, Moz, Ken, Tan, ext. to Gui			AY730110[b]	AY730199[b]
Ficus tremula Warb.	N Ang, Zai, Rwa, Bur, ext. to SE Nig, Ken, Tan, E Moz and NE RSA			AY730111[b]	AY730200[b]
Ficus umbellata Vahl.	NW Ang, ext. to CAR and Sen	FB/S2813 (BR)	LC (BR)	DQ455644	DQ455674
Subsection *Chlamydodorae*					
Ficus faulkneriana Berg	Tan, Ken	Luke and Mbinda 5824 (K)	Kenya	DQ455645	—
Ficus amadiensis De Wild.	Cam, Zai to Tan and Ken	Bidgood et al. 3518 (K)	Tanzania	DQ455646	—
Ficus burkei (Miq.). Miq. (syn.*F. thonningii* Bl.)	Ken, Uga, Tan, south to Nam, Bot and RSA	Forest 345 (NBG)	LC (NBG), 509-77	AY730095[b]	AY730184[b]
Ficus burtt-davyi Hutch.	SE RSA, S Moz	Forest 328 (NBG)	LC (NBG), 218-83	DQ455647	DQ455675
Ficus calyptrata Vahl.	Gui to Cam and Zai	Rønsted 133 (K)	LC (BG), 88.236	DQ455648	DQ455676
Ficus craterostoma Mildbr. and Burret	Ang, Zam, Moz, Zim, RSA, ext. to Uga and Sie	Forest 340 (NBG)	LC (NBG), 46-79	AY730097[b]	AY730186[b]
Ficus fischeri Mildbr. and Burret	Ang, Zam, Moz, Zim, Bot, Nam. ext. to Tan	Rønsted 100 (K)	LC (BG), 92.378	DQ455649	—
Ficus fischeri Mildbr. and Burret (syn. *F. kiloneura* Hornby)	Ang, Zam, Moz, Zim, Bot, Nam, ext. to Tan	Forest 327 (NBG)	LC (NBG)	AY730098[b]	AY730187[b]
Ficus lingua De Wild and T. Durand	N Ang to Uga, Cam and Ivo, RSA, Moz, Mal, E Tan, E Ken			AY730099[b]	AY730188[b]
Ficus lingua ssp *lingua* De Wild (syn. *F. buxifolia* De Wild.)	N Ang and Uga to Cam, Ivo and Lib			AY730096[b]	AY730185[b]
Ficus natalensis ssp *lepreurii* (Miq.) Berg	N Ang and NW Zam through Zai and west to Sen			AY730100[b]	AY730189[b]
Ficus petersii Warb. (syn. *F. thonningii* Bl.)	Ken and Tan to E RSA			AY730101[b]	AY730190[b]
Ficus reflexa Thunb. ssp. *reflexa*	Mad, Mas	Maurin et al. 76 (K)	Madagascar	DQ455650	—
Ficus thonningii Bl.	Ang, Zam, Mal, Moz, Zim, Swa, Bot, Nam, RSA, ext. to Eth and Sen			AY730102[b]	AY730191[b]
Subsection *Crassicostae*					
Ficus elasticoides De Wild.	N Ang, ext. to Zai, Cam and Ivo	Rønsted 128 (K)	LC (BG), 94.426	AY730103[b]	AY730192[b]
Ficus oreodryadum Mildbr.	Uga, Rwa, Bur to Cam, SE Nig and Fer	Thomas 9481 (K)	Cameroon	DQ455651	—
Ficus oreodryadum Mildbr.		Rønsted 228 (K)	Cameroon	DQ455652	—
Ficus usambarensis Warb.	Tan, Zam, Mal	Wrangham G7111 (K)	Tanzania	DQ455653	DQ455677
Subsection *Cyathistipulae*					
Ficus ardisioides ssp *camptoneura* (Mildbr.) Berg	NW Zam, ext. to E Zai, SE Nig and Ivo	Léonard 2445 (K)	Congo	DQ455654	—
Ficus ardisioides ssp *camptoneura* (Mildbr.) Berg		Rønsted 222 (K)	Cameroon	DQ455655	DQ455678
Ficus conraui Warb.	NW Ang and SE Zai to NW Tan, Uga, and Nig, Ivo to Sie	Reekmans 9812 (K)	Burundi	DQ455656	—
Ficus cyathistipula Warb. ssp *cyathistipula*	E Ivo to Ken and Tan, and south to Ang and N Zam	Rønsted 219 (K)	Cameroon	DQ455657	DQ455679
Ficus cyathistipuloides De Wild.	Zai, Gab, Ivo	Rønsted 136 (K)	LC (BG), 88.239	DQ455658	—

Table 2 (*continued*)

Taxon	Distribution[a]	Voucher	Origin	ITS	ETS
Ficus cyathistipuloides De Wild.				—	AY063524[c]
Ficus densistipulata De. Wild.	N Ang to Uga and Cam	Rønsted 233 (K)	Cameroon	DQ455659	DQ455680
Ficus lyrata Warb.	Sie to Cam			AY730104[b]	AY730193[b]
Ficus preussii Warb.	Uga to Nig	Rønsted 138 (K)	LC (BG), 92.1123	AY730105[b]	AY730194[b]
Ficus preussii Warb.		Rønsted 211 (K)	Cameroon	DQ455660	—
Ficus sagittifolia Mildbr. and Burret	Gui to Cam	Chase 19852 (K)	LC (K), K1986-3423	AY730106[b]	AY730195[b]
Ficus scassellatii Pamp.	E Zim, Moz, Mal, Tan, Zai, Ken, Som	Rønsted 110 (K)	LC (BG), 88.293	AY730107[b]	AY730196[b]
Ficus scott-elliotii Mildbr. and Burret	Sen to Ivo	FB/S3759 (BR)	LC (BR)	DQ455661	DQ455681
Ficus tesselata Warb.	Sie to E and S Zai, Rwa	Rønsted 143 (K)	LC (BG), 88.281	DQ455662	DQ455682
Ficus wildemaniana Warb.	Uga to Cam			AY730108[b]	AY730197[b]
Subsection *Galoglychia*					
Ficus lutea Vahl.	RSA to Sen and Eth, Mad, Mas, Sey, Ald and Com			AY063564[c]	AY063525[c]
Ficus saussureana DC.	Gui to S Sud, W Ken, NW Tanz			AY730090[b]	AY730179[b]
Subsection *Platyphyllae*					
Ficus abutilifolia (Miq.) Miq.	Som to Gui, and in NE RSA, Zim, E Bot, W Moz, Mal, E Zam, SE Zai and SW Tan			AY730091[b]	AY730180[b]
Ficus glumosa Delile.	SW Ang, Zam, Mal, Moz, Zim, Swa, Bot, Nam, RSA, ext. N to Eth Sud and W to Sen, Yem			AY063562[c]	AY063523[c]
Ficus nigropunctata Mildbr. and Burret	Zam, Mal, Moz, Zim, N Bot, ext. to Tan and Ken	Bidgood et al. 1155 (K)	Tanzania	DQ455663	—
Ficus platyphylla Delile.	Sen to Som			AY730092[b]	AY730181[b]
Ficus platyphylla Delile		Rønsted 102 (K)	LC (BG), 92.376.	DQ455664	—
Ficus populifolia Vahl.	Gha to Eth and N Tan, Yem			AY730093[b]	AY730182[b]
Ficus tettensis Hutch.	E Bot, S-NE Zim, Moz and N RSA	Forest 337 (NBG)	LC (NBG), 913-96	DQ455665	DQ455683
Ficus trichopoda Baker	N Zam, Mal, Moz, RSA, ext. through Tan and Uga to Sud and Sen, Mad	Rønsted 118 (K)	LC (BG), 88.267	DQ455666	DQ455684
Outgroup					
Ficus albert-smithii Standley	South America	Rønsted 105 (K)	LC (BG), 89.519	AY730069[b]	AY063157[b]
Ficus americana Aubl.	South America	Rønsted 154 (K)	LC (BG), 94.0678	AY730070[b]	AY730158[b]
Ficus cestrifolia Schott	South America	Rønsted 139 (K)	LC (BG)	AY730076[b]	AY063164[b]
Ficus citrifolia Miller	South America	Rønsted 112 (K)	LC (BG), 89.537	AY730077[b]	AY063165[b]
Ficus crocata (Miq.) Miq. (syn. *F. tomentella*)	South America	Rønsted 120 (K)	LC (BG), 88.259	DQ455667	DQ455686
Ficus drupacea Thunb.	Asia and Australasia	Rønsted 114 (K)	LC (BG), 92.755	AY730066[b]	AY063154[b]
Ficus rubiginosa Desf.	Australia	Rønsted 89 (C)	LC (C), 1859-0014	AY063569[c]	AY063530[c]
Ficus schumacheri (Liebmann) Urban	South America	Rønsted 123 (K)	LC (BG), 88.274	AY063567[c]	AY063528[c]
Ficus subandina Dugand	South America	Rønsted 104 (K)	LC (BG), 93.1674	DQ455668	DQ455687
Ficus superba Miq.	Asia and Malesia	Rønsted 63 (C)	LC (C), S1864-0160	AF165410[d]	AY063149[b]
Ficus trigona L.	South America	Rønsted 103 (K)	LC (BG). 90.630	DQ455669	DQ455688

Classification and distribution of taxa according to Berg (1986) and Berg and Wiebes (1992).

[a] *Abbreviations used:* N, North; W, West; S, South; E, East; C, central; ext, extending; ex, except; syn., synonym; LC, living collection; Ald, Aldabra Islands; Ang, Angola; Ben, Benin; Bot, Botswana; Brk, Burkina; Bur, Burundi; Cam, Cameroon; CAR, Central African Republic; Cha, Chad; Com, Comores; DRC, Democratic Republic of Congo; Eri, Eritrea; Eth, Ethiopia; Fer, Fernando Po; Gab, Gabon; GBi, Guinea Bissau; Gha, Ghana; Gui, Guinea; Ivo, Ivory Coast; Ken, Kenya; Les, Lesotho; Lib, Liberia; Mad, Madagascar; Mal, Malawi; Mas, Mascarene Islands; Moz, Mozambique; Nam, Namibia; Ngr, Niger; Nig, Nigeria; QLD, Queensland; RSA, Republic of South Africa; Rwa, Rwanda; Sen, Senegal; Sey, Seychelles; Sie, Sierra Leone; Som, Somalia; Sud, Sudan; Swa, Swaziland; Tan, Tanzania; Tog, Togo; Uga, Uganda; Yem, Yemen; Zai, Zaire (Congo); Zam, Zambia; Zim, Zimbabwe.

[b] Rønsted et al. (2005).

[c] Jousselin et al. (2003).

[d] Weiblen (2000).

The matrix has been deposited in TreeBase (www.treebase.org). Study and matrix accession numbers = S1669 and M3021. In order to test the subsectional placement of *F. amadiensis*, *F. conraui*, *F. dryepondtiana*, *F. faulkneriana*, *F. nigropunctata*, *F. oreodryadum*, and *F. reflexa* for which only ITS could be sequenced, they were all subsequently included in the combined matrix, which was then subject to 1000 replicates of heuristic search saving only 10 trees per replicate (results not shown) and to bootstrap analysis as described below.

ITS and ETS are part of the same region of the nuclear ribosomal genome (Baldwin and Markos, 1998), and based on previous studies using ITS and ETS sequence data of *Ficus* (Jousselin et al., 2003; Rønsted et al., 2005), the two datasets were therefore directly combined into one matrix. Cladistic analyses were conducted using PAUP v. 4.0b5 (Swofford, 2002). All changes were assessed as unordered and equally weighed (Fitch parsimony; Fitch, 1971). The matrix was analysed under both maximum parsimony (MP) and maximum likelihood conditions (ML).

The MP analysis was limited due to the number of trees produced. Trees were obtained using the following protocol: (i) 1000 replicates using random taxon addition sequence were performed using the heuristic search option in PAUP v. 4.0b5 for Macintosh (Swofford, 2002) and tree bisection-reconnection branch swapping (TBR), with only 25 trees held at each step to save time by avoiding swapping on suboptimal islands; (ii) the trees collectively found in these 1000 replicates were then used as starting trees for a second search using TBR branch swapping until all trees were found or a pre-set maximum of 15,000 trees were found. Relative levels of homoplasy in all the datasets were assessed from all characters using the consistency index (CI) and the retention index (RI) as implemented in PAUP. In all cases RI was at least 0.79, which indicates that only one island of trees was likely to exist (Maddison, 1991). Section *Galoglychia* was paraphyletic to section *Americana* in all trees produced by the combined analysis of ITS and ETS (see results below). In order to investigate this further, we constrained *Galoglychia* as monophyletic and repeated the analysis. One of the trees from the unconstrained analysis was imported into MacClade (Maddison and Maddison, 2001), section *Americana* was placed as sister to section *Galoglychia* and all other ingroup and outgroup branches were collapsed. This tree was then used as a topological constraint in a second MP analysis in PAUP. We then used the Templeton test (Templeton, 1983) to compare the length of the constrained and the unconstrained trees.

For the ML analysis, we first selected the best fitting model using Modeltest (Posada and Crandall, 1998). The model parameters thus obtained were then used for 100 replicates of heuristic search under the ML criterion.

Clade robustness was assessed using both Bayesian analysis and non-parametric bootstrap re-sampling. The Bayesian analysis was performed using MrBayes 3.1.2 (Hulsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). We used an HKY85 model of evolution (lset

NST = 2 RATES = equal). The analysis was performed with two independent runs of 10 million generations on four Monte Carlo Markov chains with equal rates and trees sampled every 1000 generations (mcmc NGEN = 10,000,000, PRINTFREQ = 10,000, SAMPLEFREQ = 1000, NCHAINS = 4). The first 1000 trees of low posterior probability were deleted, and all remaining trees were imported into PAUP. A majority rule consensus tree was produced showing the posterior probabilities (PP) of all observed bi-partitions. We also generated the 95% of credible trees with MrBayes 3.1.2 to investigate the monophyly of section *Galoglychia* in further detail.

Bootstrap support (Felsenstein, 1985) was assessed using 1000 replicates each consisting of 10 random addition sequence replicates with TBR swapping and no limits on the number of trees saved. We defined scores between 50 and 74 bootstrap percentages (BS) as weak support, scores between 75% and 89% BS as moderate support, and scores of 90% BS or more as high support. We consider percentages <50% to be unsupported because such groups often are not present in all shortest Fitch trees. We show only scores greater than 50% BS that are consistent with the strict consensus tree.

3. Results

3.1. Is section Galoglychia monophyletic?

The aligned combined ITS + ETS matrix contained 1,344 base pairs of which 303 (23%) were variable and 129 (10%) were potentially parsimony informative. The combined analysis of ITS + ETS produced 15,000 trees of length 461. One of the trees is shown in Fig. 1 with bootstrap support and Bayesian posterior probabilities shown above the branches. Arrowheads indicate branches that collapse in the strict consensus tree of all 15,000 trees. In all of these trees, section *Galoglychia* was paraphyletic to section *Americana*, although the bootstrap analysis showed a trichotomy of two clades with members of section *Galoglychia* (clades A and B) and a clade with section *Americana*. In order to investigate the monophyly of section *Galoglychia* further, we constrained *Galoglychia* as monophyletic and repeated the analysis. The constrained analysis produced 8256 trees, which were only one step longer (462 steps, CI = 0.73, and RI = 0.82) and were not significantly different from the 15,000 trees obtained by the unconstrained combined analysis (*p* = 0.5637). Maximum likelihood analysis produced two most likely trees (log likelihood = 4794.96). One of the trees is shown if Fig. 2. An arrowhead indicates the single branch that collapses in the strict consensus tree of the two trees. In both trees, section *Galoglychia* is paraphyletic to section *Americana*. However in the ML trees, section *Americana* is sister to clade B of section *Galoglychia*, whereas it was sister to clade A of section *Galoglychia* in the MP analysis. Bayesian analysis produced the same overall topology (data not
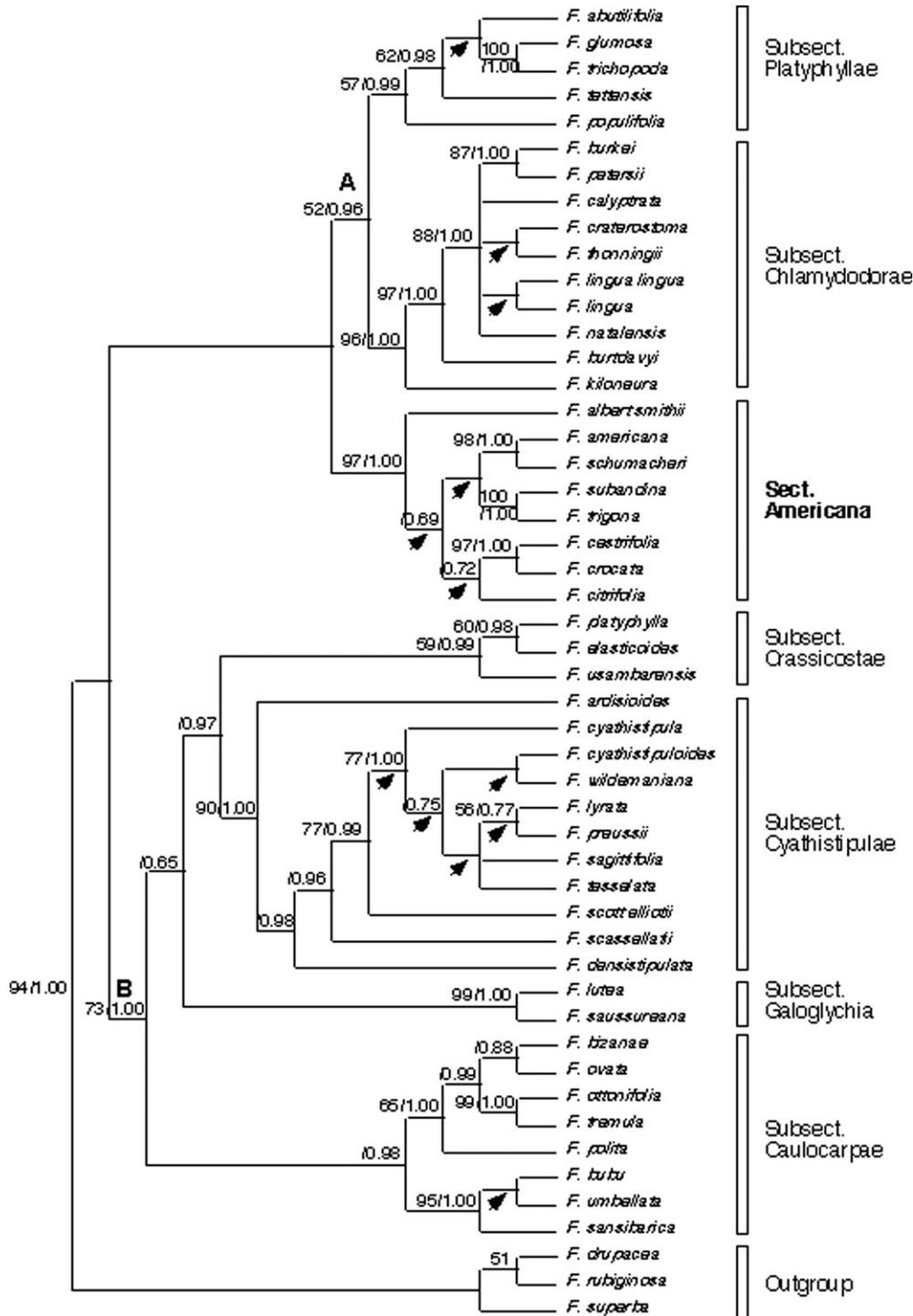
Fig. 1. One of 15,000 most parsimonious trees from the combined maximum parsimony analysis of nuclear ribosomal internal and external transcribed spacers (ITS and ETS) of *Ficus* sections *Galoglychia* and *Americana*. Length = 461 steps, consistency index = 0.73, retention index = 0.82. Bootstrap percentages/Bayesian posterior probabilities are shown above branches. Arrowheads indicate branches that collapse in the strict consensus tree.

shown) as the ML analysis did (Fig. 2). Bayesian analysis resolved a number of branches that were not resolved in the ML analysis, but all of these only have a posterior probability of 0.65 or less (probabilities are between 0 and 1). The Bayesian tree is not shown, but the posterior probabilities obtained are indicated on the MP and ML trees shown in Fig. 1 and Fig. 2. The sister relationship of

section *Americana* and clade B, found in both the Bayesian and the ML analyses, has a posterior probability of 0.79. The set of 95% credible Bayesian trees include 17,999 trees. 13,632 of these trees show section *Americana* as sister to clade B, 2681 trees show section *Americana* as sister to clade A, and only 1304 of the credible show section *Galoglychia* as monophyletic.
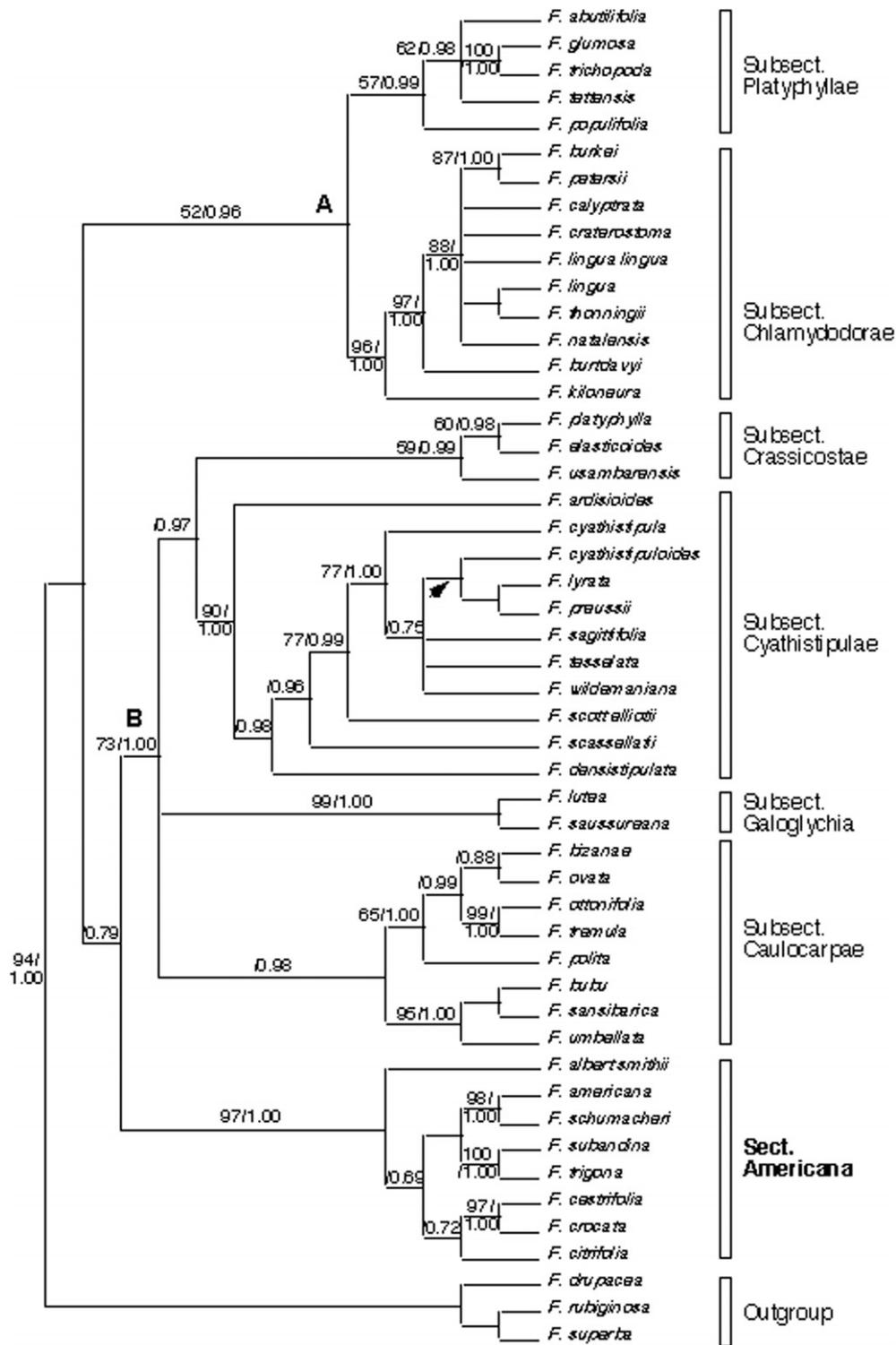
Fig. 2. One of two most likely trees (log likelihood = 4794.96) from the analysis of the combined maximum likelihood analysis of nuclear ribosomal internal and external transcribed spacers (ITS and ETS) of *Ficus* sections *Galoglychia* and *Americana*. Arrowheads indicate branches that collapse in the strict consensus tree. Bootstrap percentages (maximum parsimony)/Bayesian posterior probabilities are shown above branches.

3.2. Infrasectional relationships of section Galoglychia

The subsectional division of section *Galoglychia* proposed by Berg (1986) and Table 1 is generally supported. However, some species listed by Berg and Wiebes (1992) as difficult to place, such as *F. jansii* Boutique (putatively placed in subsection *Platyphyllae*, but could belong to subsection *Crassicostae*), and *F. burretiana* Hutch. (putatively placed in subsection *Crassicostae*, but could belong to subsection *Chlamydodorae*) were not available for this study.

We find two major clades with members of section *Galoglychia*, clades A and B. Section *Americana* is sister to clade A in the MP analysis, but to clade B in the ML and the Bayesian analyses. See Section 3.1 for further discussion.

Clade A (52% BS, PP = 0.96) includes subsections *Platyphyllae* excluding *F. platyphylla* (57% BS, PP = 0.99) and *Chlamydodorae* (96% BS, PP = 1.00) as sisters. Clade B includes the remainder of the subsections (73% BS, PP = 1.00). Subsections *Galoglychia* (99% BS, PP = 1.00) and *Cyathistipulae* (90% BS, PP = 1.00) are monophyletic and strongly supported. Subsection *Caulocarpae* (<50% BS, PP = 0.99) is also monophyletic, but not supported by the bootstrap. *Ficus platyphylla* (subsection *Platyphyllae*) is included in subsection *Crassicostae* (59% BS, PP = 0.99), but this is also only weakly supported by the bootstrap. The relationship of the subsections within clade B is uncertain (<50% BS), but subsections *Crassicostae* and *Cyathistipulae* are sisters with high posterior probability (PP = 0.97).

When the taxa for which only ITS was sequenced, were included in the combined analysis, the expected placement of *F. amadiensis*, *F. faulkneriana* and *F. reflexa* in subsection *Chlamydodorae*, *F. dryepondtiana* in subsection *Caulocarpae*, and *F. conraui* in subsection *Cyathistipulae* received low to moderate support. *Ficus oreodryadum* (subsection *Crassicostae*) was included in subsection *Cyathistipulae* with moderate support. *Ficus nigropunctata* (subsection *Platyphyllae*) was included in subsection *Chlamydodorae*, but this was not supported by the bootstrap.

Duplicate accessions of taxa appear as sister taxa, except for one of the accessions of *F. preussii*, which was only sequenced for ITS, and is sister to *F. lyrata* (55% BS) when included in the analysis, and *F. craterostoma* being sister to *F. thonningii*, although this is not supported by the bootstrap.

4. Discussion

4.1. Systematics of sections Galoglychia and Americana

Although all three types of analysis (MP, ML and Bayesian) suggest that section *Galoglychia* is paraphyletic to section *Americana*, this is not supported by the bootstrap and only has a posterior probability of 0.79. In the MP analysis, section *Americana* is sister to clade A, whereas it is sister to clade B in the ML and the Bayesian analysis. A number of simulation studies have shown that Bayesian analysis tend to overestimate the support of clades (e.g. see Simmons et al., 2004), and some studies have also shown conflicts between Bayesian and bootstrap support (Suzuki et al., 2002; Wilcox et al., 2002). In the present analyses, there also are examples of short branches having high Bayesian posterior probability. For example the branch supporting subsections *Crassicostae* and *Cyathistipulae* in clade B is only one step long in the MP analysis, but has a posterior probability of 97 in the Bayesian analysis.

In the presence of conflicts between Bayesian and bootstrap support, we follow the conservative approach of Simmons et al. (2004), who advocate the use of bootstrap and jackknife approaches to estimate support rather than the possible overestimates of Bayesian support. The conflicting results between the MP analysis and the ML and Bayesian analysis with respect to the placement of section *Americana* could be explained by lack of sufficient information in the ITS + ETS dataset. Accordingly, we conclude that section *Galoglychia* is most likely paraphyletic to the neotropical section *Americana*, although this is not supported by bootstrap analysis and only weakly supported by Bayesian posterior probabilities. A close relationship of sections *Galoglychia* and *Americana* was suggested by Mildbraed and Burret (1911), who hypothesised that section *Galoglychia* could have developed within section *Americana*. Both sections have two basal bracts, but in section *Americana*, the ostiolar bracts are interlocking instead of descending. The placement of section *Americana* with respect to the two clades of section *Galoglychia* must be considered uncertain based on the conflict between the various types of analysis in the present study. There are no obvious morphological characters supporting section *Galoglychia* clades A and B or the sister relationship between either *Galoglychia* clade A and section *Americana*, or *Galoglychia* clade B and section *Americana*. Further data will be needed to unambiguously determine the circumscription of section *Galoglychia* and the origin of section *Americana*. In the meantime we prefer not to revise the circumscription of section *Galoglychia* and this is also reflected in the following discussion of the infrasectional classification of *Galoglychia*, which focuses on the results of the MP analysis.

4.2. Towards a natural classification of figs within section Galoglychia

4.2.1. Section Galoglychia Clade A. Subsections Chlamydodorae and Platyphyllae

Subsection *Chlamydodorae* as defined by Berg (1986; Berg and Wiebes 1992) comprises thirteen species and is best represented in eastern Africa. Some species occur in rainforest and others grow in savannah woodland. Berg and Wiebes (1992) adopted the circumscription of subsection *Chlamydodorae* from Mildbraed and Burret (1911), with only a single addition of *F. fischeri*, which was previously placed in subsection *Fasciculatae*. The 10 samples included in our combined analysis, representing seven of the 13 species included in the subsection by Berg and Wiebes (1992), form a strongly supported clade. *Ficus amadiensis*, *F. faulkneriana* and *F. reflexa* were only sequenced for ITS, but they are placed in the subsection *Chlamydodorae* clade when included in the combined matrix, further supporting the monophyly of this subsection. In the combined analysis, *F. fischeri* is included in subsection *Chlamydodorae* with strong support, as is also *F. kiloneura*, which can be considered a synonym of *F. fischeri*, confirming Berg's (1986) transfer.

Within subsection *Chlamydodorae*, a number of entities (for examples, *F. burkei*, *F. persifolia* Welv. ex. Warb.,

F. petersii, F. psilopoga Welv. ex. Ficalho, *F. rokko* Warb & Schweinf., and *F. thonnigii*, along with almost 40 possible synonyms) have been united under the problematic *F. thonningii*-complex by Berg (Berg and Wiebes, 1992; van Greuning, 1990). The original description of *F. thonningii* was very brief and the type was lost under the British bombardment of Copenhagen in 1807 (Burrows and Burrows, 2003). It is therefore debateable what should be circumscribed under *F. thonningii*. Berg and Wiebes (1992) decided to list 10 forms of *F. thonningii* based on differences in their distribution, ecology and morphology, but other authors prefer to recognize a number of separate species from the *F. thonningii* complex (Burrows and Burrows, 2003). In the present study, we included samples referred to *F. thonningii*, as well as to *F. burkei* and *F. petersii*, which are both considered synonyms of *F. thonningii* by Berg and Wiebes (1992). Despite our limited sampling of the *F. thonningii*-complex, our data does not support the monophyly of the complex. *Ficus burkei* and *F. petersii* are sisters (87% BS), but *F. thonningii* is more closely related to *F. craterostoma* than to the two former, although this is not supported by the bootstrap. Further studies with extensive sampling and more variable markers will be needed to sort out this complex.

Subsection *Platyphyllae* as defined by Berg (1986; Berg and Wiebes 1992) comprises 18 species, concentrated in Eastern Africa and represented in Madagascar and neighbouring islands (Mascarene Islands, Seychelles, Aldabra Islands and Comoro Islands). Most species of this section are associated with savannah woodland and show adaptations to dry habitats. For example, *F. abutilifolia, F. glumosa, F. tettensis* and *F. muelleriana* C.C. Berg are often found on rocks, and *F. trichopoda* grows in marshy areas. Only *F. recurvata* De Wildeman and *F. jansii* are confined to the rainforest (Berg, 1986; Berg and Wiebes, 1992). Berg and Wiebes (1992) entirely adopted the circumscription of subsection *Platyphyllae* originally proposed by Mildbraed and Burret (1911). Eight species were included in the present study, five of which form a clade, whereas two accessions of *F. platyphylla* appear to be associated with members of subsection *Crassicostae*. However, there is no obvious morphological character to support the latter. Berg and Wiebes (1992) suggested that *F. jansii*, which is not included in our study, may also belong to subsection *Crassicostae*, where it has affinity to *F. adolfi-friederici* Baker. *Ficus nigropunctata* was only sequenced for ITS. Although only weakly supported (58% BS), its placement outside of subsection *Platyphyllae* further suggests that this subsection is not monophyletic as currently circumscribed.

Hutchinson (1915) united subsections *Chlamydodorae, Crassicostae*, and *Platyphyllae* in his subseries *Cauducae*. Lebrun (1934) alternatively sunk subsection *Crassicostae* into subsection *Chlamydodorae*. Subsections *Platyphyllae* and *Chlamydodorae* are closely related according to our results, but subsection *Crassicostae* appears to be more closely related to the remainder of the subsections contrasting Hutchinson's (1915) and Lebrun's (1934) classifications.

4.2.2. Section Galoglychia clade B. Subsections Caulocarpae, Crassicostae, Cyathistipulae and Galoglychia

Subsection *Caulocarpae* as defined by Berg (1986; Berg and Wiebes 1992) includes 11 species broadly distributed throughout Africa, one of which, *F. polita*, extends to Madagascar. Most species are associated with dry evergreen forest, except *F. sansibarica*, which extends to savannah woodland. Subsection *Caulocarpae* as defined by Berg (Berg, 1985; Berg, 1986; Berg and Wiebes, 1992) includes Mildbraed and Burret's (1911) sections *Caulocarpae, Fasciculatae* (except *F. fischeri*) and *Elegantes* (except *F. usambarensis*), an interpretation largely followed by Hutchinson (1915), plus *F. ottonifolia* (transferred from subsection *Crassicostae*), and *F. ovata* and *F. bubu* (formerly placed in subsection *Cyathistipulae*). Nine species of subsection *Caulocarpae* were included in our study, although two accessions of *F. dryepondtiana* were only sequenced for ITS. In the combined analysis, the taxa currently assigned to subsection *Caulocarpae* form a clade, although this is not supported by the bootstrap. When the ITS sequences of *F. dryepondtiana* is included in the analysis, it is also placed within this clade, again not supported by the bootstrap. *Ficus ovata* was added to this subsection by Berg (Berg et al., 1985; Berg, 1986), based on similarities of the fruitlets. However, *F. ovata* differs from the rest of the group in a number of other features, such as the presence of axillary syconia, that are enclosed in calyptrate buds when young, rather than caulocarpous syconia, and leaves that are occasionally densely puberulous beneath. Both accessions of *F. ovata* are well embedded in this clade, confirming that this species indeed belongs to subsection *Caulocarpae*. Berg's transfer of *F. bubu* and *F. ottonifolia* to subsection *Caulocarpae* is likewise supported by our analysis. Berg et al. (1985) recognized three informal subgroups within subsection *Caulocarpae* based on the position and surface structure of the syconia and the shape of the leaf base: (i) *F. ovata*, (ii) *F. umbellata, F. bubu* and *F. polita*, and (iii) the remainder of the species in this subsection. However, this subdivision is not supported by the present analysis. *Ficus bubu* and *F. umbellata* form a clade with *F. sansibarica* (95% BS), whereas *F. ovata* and *F. polita* form a clade with *F. bizanae, F. ottonifolia* and *F. tremula* (65%).

Subsection *Galoglychia* as defined by Berg (1986; Berg and Wiebes 1992) comprises only three rainforest species. *Ficus saussureana* and *F. chlamydocarpa* Mildbr. & Burret are found in Central and Western Africa, while *F. lutea* extends to east Africa, Madagascar and the Seychelles. All three species were placed in subsection *Cyathistipulae* by Mildbraed and Burret (1911), but our analysis clearly show that they are part of a well supported subsection *Galoglychia* (99% BS), although *F. chlamydocarpa* was not included in the present study.

Subsection *Cyathistipulae* as defined by Berg (1986; Berg and Wiebes 1992) includes 19 species occurring in Central and Western Africa, except *F. scassellatii* Pamp., which is restricted to Eastern Africa. The subsection as circumscribed by Berg (1986) includes only about half of the taxa

placed there by Mildbraed and Burret (1911). The rest of the former members of the subsection were transferred to the newly circumscribed subsection *Galoglychia*, *F. oreodryadum* to subsection *Crassicostae*, and *F. bubu* and *F. ovata* to subsection *Caulocarpae*. Subsection *Cyathistipulae* in Berg's sense (Berg, 1986) is well-defined by having coriaceous, glabrous, shortly petiolate leaves, large syconia and long, often (sub)persistent stipules. The subsection is well supported in our analyses (90% BS) and includes *F. oreodryadum*, sequenced only for ITS. The latter was provisionally excluded from subsection *Cyathistipulae*, and transferred to subsection *Crassicostae* by Berg (1986), although he was unable to examine critical characters, like those of fruitlets, to be studied on fresh material (Berg and Wiebes, 1992 p. 133). The transfer of *F. barteri* Sprague and *F. crassicosta* Warb. could not be tested due to lack of material. Berg et al. (1985) recognized two informal groups based on a suit of characters, (i) the *F. lyrata* group including *F. crassicosta*, *F. lyrata*, *F. oresbia* C.C. Berg, *F. sagittifolia*, *F. subsagittifolia* Mildbr. ex C.C. Berg, and *F. wildemaniana*, and (ii) the *F. conraui* group including the rest of the species in this subsection, but the two groups do not form clades in our analysis.

Subsection *Crassicostae* as defined by Berg (1986; Berg and Wiebes 1992) comprises eight species confined to rainforests in Central and West Africa, except the East African *F. usambarensis*. Berg's substantially modified subsection *Crassicostae* only retained *F. adolfi-friedrici* Mildbraed from the original circumscription, the rest of the species were transferred to subsections *Cyathistipulae* and *Caulocarpae*. *Ficus usambarensis* and *F. oreodryadum* were transferred to subsection *Crassicostae* from section *Elegantes* and section *Cyathistipulae*, respectively. However, Berg (1986) considered the relationship of the species placed in section *Crassicostae* unclear. Only three species were included in this study. *Ficus elasticoides* and *F. usambarensis* form a clade with *F. platyphylla*, although the placement of the latter species here is not consistent with morphology and its preference for drier habitats, and we prefer not to assign this species to a subsection until further molecular and morphological studies have confirmed its placement. This clade is sister to section *Cyathistipulae*, the members of which are also distinctly associated with rainforests, primarily in Central and West Africa (Berg, 1986).

4.3. Biogeography of African figs

African tropical forests are known to have fewer species than those of Asia and South America (Jenkins and Hamilton, 1992). Africa's climate has fluctuated a lot over the course of history, which makes it difficult to interpret the history and origin of the present day flora. During periods of global cooling, Africa was subjected to climatic fluctuations involving a succession of more humid and arid phases. In the late Quaternary aridity became more severe resulting in decline of forested areas and possibly extinctions (Hamilton, 1992; Jenkins and Hamilton, 1992). There is little evi-

dence of mass extinctions, but macrofossil and pollen profiles from East Africa show a decline in the extent of forests over the past 20 million years (MYR; e.g. Yemane et al., 1985). Present day tropical forests in the African region can be divided into three main blocks, the Central and West African area, the East African coastal forests, and Madagascar. According to Jenkins and Hamilton (1992) Madagascar separated from mainland Africa around 160 Myr, and the East African forests have probably been isolated from the Central and Western African forests for at least 500.000 years.

Rønsted et al. (2005) calibrated a phylogenetic tree of *Ficus* using both non-parametric rate smoothing and penalised likelihood methods to account for deviations from a molecular clock, and 60 Myr old fossil achenes to constrain the origin of *Ficus*. Their sampling of sections *Galoglychia* (25 taxa sampled) and *Americana* (24 taxa sampled) was more limited than in the present study. However, they obtained an age estimate of 40 Myr for the origin of section *Galoglychia*. According to their results, section *Galoglychia* diversified gradually, with clade A and B originating 38 Myr followed by diversification of clade B about 33 Myr and clade A about 31 Myr.

The two main clades, A and B, found in the combined analysis (Fig. 1) are concentrated in different parts of the African floristic region. Clade A comprising members of subsections *Platyphyllae* and *Chlamydodorae* is more concentrated in Eastern Africa and extends to Madagascar and neighbouring islands (Comores, Mascarenes, Aldabra Islands and Seychelles). The seven Malagasy species are not confined to one subsection or clade. Clade A includes many species, especially in section *Platyphyllae*, that are adapted to dry habitats and many of these occur in savannah woodland. Some members of clade A (e.g. *F. calyptrata*, *F. jansii*, and *F. recurvata*) are restricted to the rainforest and some (e.g. *F. natalensis* and *F. thonningii*) occur in both types of habitats (Berg and Wiebes, 1992). In a study including 1818 species (representing about 5% of the flora of sub-Saharan Africa), Linder (2001) used indices of species richness and endemism to locate six important centres of endemism in sub-Saharan Africa. Two of Linder's important areas of endemism, the East African coast from Malindi to Mozambique, and the watershed of the Zambezi and Congo rivers, are found in Eastern Africa.

Clade B includes members of subsections *Caulocarpae*, *Cyathistipulae*, *Crassicostae* and *Galoglychia*, which are primarily concentrated in West and Central Africa, and a majority is associated with lowland forests of the Guinea-Congolian region (Berg, 1990a,b). The Congolean forest is far more extensive than any other forested area in Africa and contains a considerably greater number of species (an estimated 8000 plant species of which 80% are endemic; Jenkins and Hamilton, 1992). It is also the *Ficus* hotspot of mainland Africa. Of the circa 110 species of *Ficus* in the African floristic region, 60 are found in Cameroon and 40 or more in Gabon (Berg et al., 1984, 1985). Many of the species are shared between the two regions, but Gabon is

less explored and does not have the species adapted to drier habitats such as the savannah (Berg et al., 1984, 1985). Of the 72 species belonging to section *Galoglychia*, 45 are found in Cameroon alone (Berg et al., 1984, 1985; Berg and Wiebes, 1992). The Guineo-Congolean region includes the Lower Guinea centre of endemism, which is also one of the six important areas of endemism and species richness in sub-Saharan Africa identified by Linder (2001).

Acknowledgments

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