

Evolution and biodiversity of the Ixoroideae
(Rubiaceae)

Kent Kainulainen



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Cover illustration: *Nematostylis* sp. nov., Marojejy National Park, Madagascar.

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Abstract

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The phylogenetic relationships within subfamily Ixoroideae of the coffee family are investigated by phylogenetic reconstruction of molecular data, including regions of the chloroplast DNA (*matK*, *ndhF*, *rbcL*, *rps16*, *trnH-psbA*, *trnS-G*, and *trnT-F*), and the nuclear ribosomal DNA (ITS). The evolution of morphological characters within the group are inferred, with focus on characters used in classification. Ixoroideae have primarily been characterized by secondary pollen presentation, contorted corolla aestivation, and fleshy fruits. Secondary pollen presentation appears synapomorphic of a clade comprising the Ixoroideae crown group together with *Retiniphyllum*, whereas contorted corolla aestivation has evolved earlier and is synapomorphic for the crown group, *Retiniphyllum*, and *Steenisia*. Capsules likely represent a plesiomorphy from which various dry or fleshy indehiscent fruits have evolved independently in different clades. Reductions in seed number have also occurred in many clades, none of which shows a secondary increase in the number of seeds.

Within Ixoroideae, the phylogeny and tribal delimitations of Alberteae and Condamineae are studied in more detail. The former appears restricted to *Alberta*, *Nematostylis*, and *Razafimandimbisonia*, a new genus described here. The Condamineae are a diverse tribe largely unresolved in previous molecular phylogenetic studies. Our results support a synonymization of both Calycophylleae and Hippotideae, because these are nested within the Condamineae. Ancestral state reconstructions indicate that intrapetiolar stipules, poricidal anthers, and protogyny, otherwise uncommon characters in Rubiaceae, all have evolved more than once in the Condamineae.

The rare genera *Jackiopsis*, *Glionnetia*, and *Trailliaedoxa* previously not included in molecular phylogenetic analyses, are all found nested within the Ixoroideae, and their systematic positions are discussed. The genera *Bathysa*, *Calycophyllum*, *Elaeagia*, and *Rustia* do not appear monophyletic. Consequently, resurrections of the names *Holtonia*, *Schizocalyx*, and *Semaphyllanthus*, and synonymizations of *Phitopsis* (as *Schizocalyx*) and *Tresanthera* (as *Rustia*) are proposed. Also proposed are five new tribal names for clades that are not associated with any previously described tribes in the phylogenetic hypotheses presented.

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Preface

This thesis is based on the following papers, referred to in the text by their Roman numerals.

- I Kainulainen, K., Mouly, A., Khodabandeh, A., and Bremer, B. 2009. Molecular phylogenetic analysis of the tribe Alerteae (Rubiaceae), with description of a new genus, *Razafimandimbisonia*. *Taxon* 58: 757–768.
- II Kainulainen, K., Persson, C., Eriksson, T., and Bremer, B. 2010. Molecular systematics and morphological character evolution in the Condamineae (Rubiaceae). *American Journal of Botany* 97: 1961–1981.
- III Razafimandimbison, S. G., Kainulainen, K., Khoon, M. W., Beaver, K., and Bremer, B. Molecular support for a basal grade of morphologically peculiar, monotypic genera in the species-rich Vanguerieae alliance (Rubiaceae): its systematic and conservation implications. Submitted.
- IV Kainulainen, K., Razafimandimbison, S. G., and Bremer, B. Phylogeny and evolution of secondary pollen presentation, corolla aestivation patterns, fruit types, and seed number in the Ixoroideae (Rubiaceae). Manuscript.

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Disclaimer: It is not the authors intention that the tribal names described in Paper IV should be validly published in this thesis, therefore the latin diagnoses have been omitted.

Papers I, II, and IV, were written by KK, with comments and suggestions given by the co-authors. The papers were planned in cooperation with co-authors. KK is responsible for the analyses of Paper I and IV, the character optimization and parsimony analyses of Paper II, and the analyses of the combined datasets in Paper III. KK contributed to the writing of Paper III, and is responsible for most lab work in all papers.

Contents

Introduction	11
Material and Methods.....	13
Results and Discussion.....	15
Svensk sammanfattning.....	19
Acknowledgements.....	22
References	23

Abbreviations

c.f.	confer, compare
DNA	deoxyribonucleic acid
et al.	et alii, and others
i.e.	id est, that is
ITS	internal transcribed spacer
matK	maturase K
ndhF	nadh dehydrogenase subunit F
psbA	photosystem II protein D1
rps16	ribosomal protein S16
SEM	scanning electron microscope
s.l.	sensu lato, in the wide sense.
s.s.	sensu stricto, in the strict sense.
trn	transfer ribonucleic acid
vs.	versus

Introduction

This thesis deals with the systematics of Ixoroideae, a subfamily of the Rubiaceae. The family Rubiaceae is part of the angiosperm order Gentianales (APG, 1998). In terms of species number it is the 4th largest plant family. The Rubiaceae are morphologically very diverse, and range from small annual herbs such as *Limnosipanea* to tall canopy trees such as *Chimarrhis*. Still, they can mostly be easily recognized by a combination of opposite, simple leaves, presence of stipules, and inferior ovaries. Rubiaceae are mainly distributed in the tropics, with relatively few representatives in temperate regions.

Systematics includes both the study of the evolution of organisms (phylogenetics), and their classification (taxonomy). Taxonomy should reflect phylogeny (Hennig, 1966; Farris, 1979), i.e. in a meaningful classification only monophyletic (Haeckel, 1866) or natural groups are formally recognized. In contrast, traditional systems of classification of the Rubiaceae (Hooker, 1873; Schumann, 1891) relied mostly on single key characters (most notably number of ovules, fruit types [dry or fleshy], and absence/presence of seed wings) for taxonomic groupings, in the sense that the classification was an extension of the key. The distinction of classification vs. key, was further stressed by more recent authors (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988), who proposed improvements in classification by the consideration of different morphological, biochemical, and cytological characters in combination. Special emphasis was placed on characters considered of greater taxonomic importance. In their respective re-evaluations of the Rubiaceae classification, the varying emphasis of character importance made by these authors resulted in different numbers of recognized subfamilies, and somewhat different placement of certain problematic tribes.

The subfamily Ixoroideae, focus of this thesis, was described by Bremekamp (1966) as including taxa with secondary pollen presentation, a character considered of high taxonomic value as it required a combination of characters to be functional. His circumscription comprised the tribes Acranthereae, Chiococceae, Coptosapelteae, Cremasporeae, Gardenieae, Ixoreae and Vanguerieae (the correct name of Ixoreae as defined by the

author should be Coffeae; Darwin, 1976). Robbrecht (1988), limited Ixoroideae to tribes with contorted corolla aestivation and fleshy fruits. Included were tribes corresponding to Cremasporae, Gardenieae, and Ixoreae *sensu* Bremekamp (Aulacocalyceae, Coffeae, Gardenieae, Octotropideae, and Pavetteae).

The development of methods of phylogenetic reconstruction (Fitch, 1971; Felsenstein, 1981; Yang and Rannala, 1997), and the introduction of DNA sequence data into systematics (Ritland and Clegg, 1987; Woese, 1987), were instrumental in the development of molecular systematics, the application of which has resulted in extensive changes in classification for many groups of organisms, including the Rubiaceae. Early molecular phylogenetic studies based on *rbcL* data (Bremer et al., 1995; Andreasen and Bremer, 1996), supported the Ixoroideae *sensu* Robbrecht (1988; although also including the Vanguerieae). However, several taxa from tribes previously placed in the Cinchonoideae also appeared to be associated with the subfamily. Consequently, Andreasen (1997) proposed an expansion of the Ixoroideae to include these basal clades. Subsequent studies by Bremer and Thulin (1998), Bremer et al. (1999), and in particular by Rova (1999), included larger samples from these clades (i.e. representatives of the Condamineae, Mussaendeae, Posoquerieae, Sabiceae and Sipaneeae), and in all these studies the authors argued that these clades were best considered as part of the Ixoroideae. However, this expanded Ixoroideae (s.l.) could no longer be easily characterized morphologically.

The relationships within the Ixoroideae (s.s.) were assessed by Andreasen and Bremer (2000), who also provided revised delimitations of the Coffeae, Cremasporae, Gardenieae, Ixoreae, and Pavetteae. Since then, the phylogenetic relationships of several of these and other tribes of the subfamily have been further investigated by molecular phylogenetic methods; Coffeae (Davis et al., 2007); Gardenieae (Persson, 2000); Ixoreae (Mouly et al., 2009); Mussaendeae (Alejandro et al., 2005); Sabiceae (Dessein et al., 2001; Kahn et al., 2008); Retiniphyllae (Cortés-B et al., 2009); Sipaneeae (Delprete and Cortés-B, 2004); and Vanguerieae (Lantz and Bremer, 2004, 2005; Razafimandimbison et al., 2009).

Andreasen (1997) found the tribe Alberteae nested within the Ixoroideae, although with an uncertain position. The position and delimitation of this tribe is the focus of paper I. In paper II we investigate the largely unresolved phylogeny of the clade consisting of Condamineae and associated genera. Paper III deals with the phylogenetic positions of the enigmatic genera *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa*, and finally, in paper IV we infer hypotheses of phylogeny and morphological character evolution of the Ixoroideae.

Material and Methods

These studies were primarily based on phylogenetic reconstruction from DNA sequence data. The taxon sampling in paper I included DNA data from several specimens of *Alberta* and *Nematostylis*. Sampled were also *Airosperma*, *Aulacocalyx*, *Boholia*, and *Crossopteryx* (genera previously suggested as possibly associated with Alberteae), and representatives of most tribes of the Ixoroideae. The DNA regions used were ITS, rbcL, ndhF, rps16 (intron), trnH-psbA, trnS-G, and trnT-F. The study of the Condamineae complex (paper II), included all the genera associated with the group in previous molecular studies, as well as the genera *Phitopsis*, *Tammsia*, and *Tresanthera*. Additional species were included for genera that did not appear monophyletic in preliminary analyses (*Bathysa*, *Elaeagia*, and *Rustia*). The ingroup comprised 71 species representing 32 genera. The DNA regions used were ITS, matK, ndhF, rps16, trnH-psbA, trnS-G, and trnT-F. For paper III, three specimens of *Glionnetia* and *Jackiopsis*, and two specimens of *Trailliaedoxa*, were added to a in terms of taxon sampling somewhat expanded version of the dataset of paper I. The DNA regions ndhF, rbcL, rps16, and trnT-F were included. In paper IV, we included samples representing all tribes inferred as part of Ixoroideae in previous studies. The ingroup comprised 110 taxa from 87 genera, and the DNA regions matK, ndhF, rbcL, rps16, trnS-G, and trnT-F were used.

Methods of phylogenetic reconstruction included parsimony and Bayesian inference. Parsimony analyses were done using PAUP* 4.0b10 (Swofford, 2002), and clade support was estimated by bootstrap analysis (Felsenstein, 1985). Bayesian inference analyses were done in MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), using nucleotide substitution models as suggested by MrModeltest2 (Nylander, 2004; for paper I) or MrAIC (Nylander, 2004b; for papers II-IV).

The analyses of character evolution in paper II and paper IV, were done using the program Mesquite (Maddison and Maddison, 2010). Ancestral state reconstructions were calculated across a tree sample in order to take into account uncertainty in the tree topology. Reconstructions were made with a likelihood criterion and using the implemented Mk1 model (in which

character transitions are equally probable irrespective of direction). Probabilities were summarized on the sampled tree with the highest marginal likelihood.

Morphological information was mostly obtained from literature, complemented with observations of herbarium material, and studies of flowers and seeds using a stereo microscope. Paper II also included SEM studies of seeds.

Results and Discussion

In paper I, the position and delimitation of Alberteae is investigated. In the phylogenetic hypotheses presented, the Alberteae are resolved as sister group to the clade comprising the tribes Bertiereae, Coffeae, Cremasporeae, Gardenieae, Octotropideae, and Pavetteae. Neither *Airosperma*, *Aulacocalyx*, *Boholia*, nor *Crossopteryx* are associated with the tribe as has previously been suggested (Robbrecht, 1988; Robbrecht and Manen, 2006). Furthermore, *Alberta* appears paraphyletic. The Malagasy species form a sister clade to *Nematostylis*. We consider the former clade as best recognized at generic level, and propose the new genus *Razafimandimbisonia*, characterized by schizocarpous fruits, and anthers lacking basal appendages (unlike *Alberta* and *Nematostylis*, plants of *Razafimandimbisonia* also appear to be aluminium accumulators; cf. Jansen et al., 2000).

As mentioned, the early divergent clades of the Ixoroideae inferred in the studies by Bremer et al. (1999) and Rova (1999, 2002) included several genera historically classified in tribes of the Cinchonoideae. One of these was a diverse but poorly resolved clade which besides the type of the Condamineae also included genera classified in (mainly) the Cinchoneae, Rondeletieae, or the Hippotideae. The relationships and morphological character evolution within the Condamineae are the subjects of paper II. Inferred character evolution indicate that interpetiolar stipules, protandry, and septicidal capsules, are ancestral character states in the Condamineae, Intrapetiolar stipules, calyprate stipules, protogyny, loculicidal capsules, and indehiscent fruits appear to have evolved several times within the tribe.

The genera *Bathysa*, *Calycophyllum*, *Elaeagia*, and *Rustia* are not monophyletic in the phylogenetic hypotheses presented. This polyphyly supports the recognition of the names *Holtonia*, *Schizocalyx*, and *Semaphyllanthe* (names previously used for the taxa that are not found associated with the respective types). Furthermore, *Phitopsis* appears nested within the *Schizocalyx* clade, and *Tresanthera* is nested within the *Rustia* clade. Consequently, proposals are made to reduce *Phitopsis* and *Tresanthera* to synonymy, and to transfer several taxa currently classified as *Bathysa* to *Schizocalyx*.

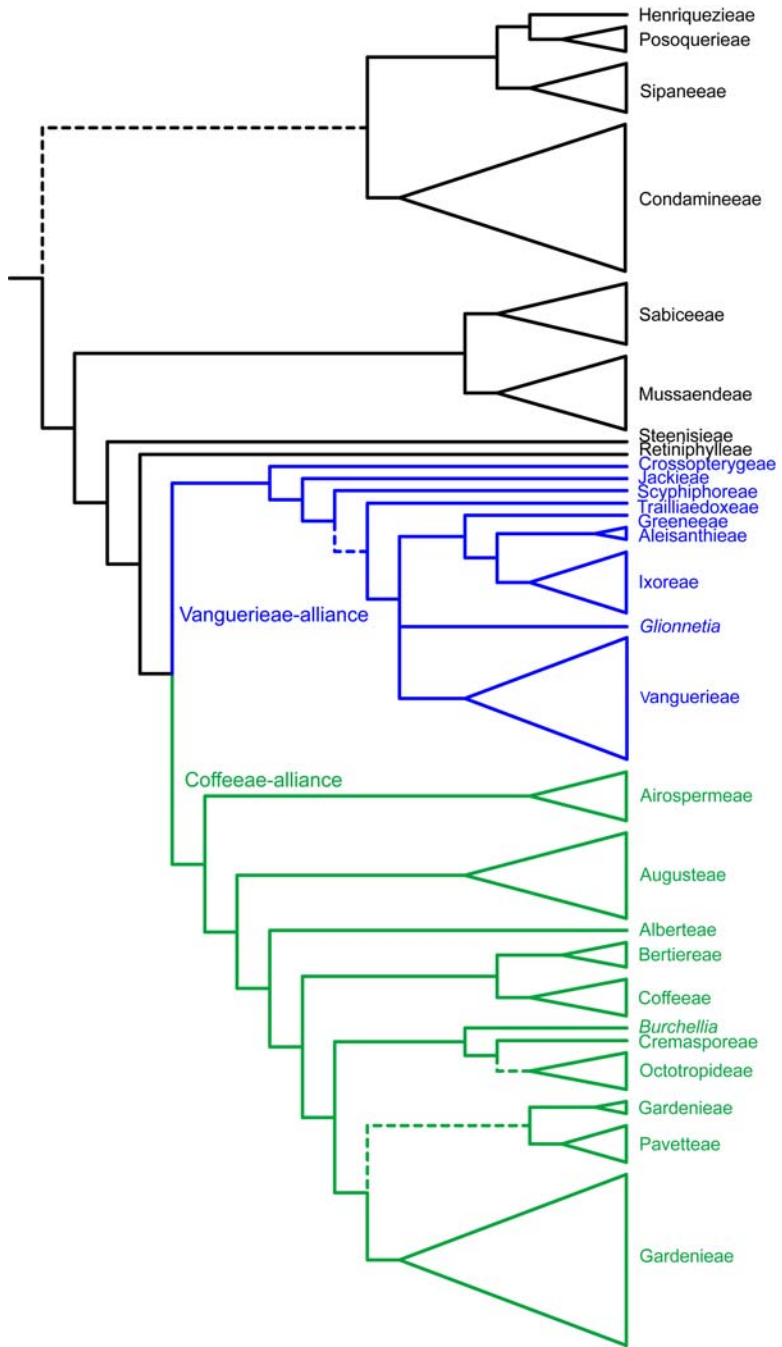


Figure 1: The phylogenetic hypothesis of Ixoroideae presented in paper IV, showing the main clades of the subfamily. Dashed clades are poorly supported.

In paper III, the phylogenetic positions of the genera *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* are investigated. As several of the genera discussed in paper II, *Glionnetia* has been included in the Rondeletieae based on its many-seeded capsules and contorted corolla aestivation (Tirvengadam, 1984; Robbrecht, 1988), whereas classifications of the morphologically somewhat aberrant *Jackiopsis* has differed widely between authors (Jackieae, Korthals, 1851; Retiniphyllae, Hooker, 1873; or Oldenlandieae, Schumann, 1891). A monogeneric Jackieae (Ridsdale, 1979), was one of the taxa that Robbrecht (1988) considered of uncertain position. Another such taxa is the little known genus *Trailliaedoxa*, by the same author considered *insertae sedis* within the Antirheoideae (a subfamily shown to be polyphyletic in subsequent molecular studies; Bremer et al., 1995).

In the phylogenetic hypotheses presented, *Glionnetia*, *Jackiopsis*, and *Trailliaedoxa* as well as *Crossopteryx* and *Scyphiphora*, all appear to represent early divergent clades within the Vanguerieae-alliance (Fig. 1). These genera are found in widely different ecosystems, i.e. African savannas (*Crossopteryx*), swamp forests of western Malesia (*Jackiopsis*), mangroves of the Indian ocean and western Pacific (*Scyphiphora*), mountain slopes of Yunnan (*Trailliaedoxa*), or the granite mountains of the Seychelles (*Glionnetia*), respectively. Ecologically conservative taxa forming (depauperate) sister groups to highly diverse clades have been suggested as characterizing phylogenetic relicts (Simpson, 1944; Brooks and Bandoni, 1988). Considering this, and the ongoing destruction or narrow range of some of their habitats, we suggest that these monotypic genera should be considered of premier conservation priority (although the widely distributed *Crossopteryx* is of less concern at present).

Finally, in paper IV, we investigate the phylogeny and morphological character evolution of Ixoroideae, with focus on characters used in earlier classification. We conclude that the Ixoroideae can be considered as consisting of a crown group that includes most of the Ixoroideae *sensu* Bremekamp (except Acranthereae, Chiococceae, and Coptosapelteae; that have been resolved in other clades of Rubiaceae in previous studies; Bremer et al., 1995; Bremer et al., 1999; Rydin et al., 2009) and Ixoroideae *sensu* Robbrecht, as well as the tribes Alberteae, Aleisanthiae, Bertiereae, Crossopterygeae, Greeneae, and Jackieae, and the genera *Airosperma*, *Augusta*, *Boholia*, *Glionnetia*, *Scyphiphora*, *Trailliaedoxa*, and *Wendlandia*. The two major clades of the crown group are the Coffeae-alliance and the Vanguerieae-alliance respectively (Fig. 1). Sister group to the crown group is Retiniphyllae, followed by a successive grade of in turn *Steenisia*, *Mussaendeae* together with *Sabiceae*, and a poorly supported clade comprising the *Condamineae* as sister to the *Henriquezieae-Posoquerieae*-

Sipaneeae clade. The Gardenieae are not monophyletic, as has also been shown in earlier studies (Andreasen and Bremer, 1996; Bremer and Eriksson, 2009).

Ancestral state reconstructions of the main characters defining Ixoroideae as emphasized by Bremekamp (1966), and Robbrecht (1988), i.e. secondary pollen presentation and contorted corolla aestivation respectively, indicate that the former appears synapomorphic for the clade consisting of the crown and Retiniphyllaeae, whereas the latter is synapomorphic for the clade comprising the crown group, Retiniphyllaeae, and *Steenisia*. Although apparently of singular origin within the Ixoroideae, analogous secondary pollen presentation occurs in other groups of the Rubiaceae (*Coptosapelta*, *Phuopsis*, and Naucleaeae; Puff et al., 1996). Contorted aestivation is analogously synapomorphic of the Sipaneeae. The character states in both clades are derived from imbricate aestivation, which is inferred as the plesiomorphic state. Valvate aestivation also appears homoplastic, and occurs in Jackieae (autapomorphic), Sabiceaeae (synapomorphic), Vanguerieae (synapomorphic), Condamineaeae, and Mussaendeae (ancestral states reconstructions of the two latter tribes were equivocal).

Capsular fruits with numerous seeds most likely represents the ancestral fruit type in Ixoroideae, from which many types of dry or fleshy indehiscent fruits have evolved. The character optimization indicates that various dry indehiscent fruits have evolved independently at least 7-10 times, and fleshy fruits 7-8 times. With a possible exception within Mussaendeae (reconstruction equivocal), reversals to capsular fruits have not occurred. Notably, when comparing clades characterized by presumed actively dispersed fruits with clade of equal age (sister clades) characterized by passively dispersed fruits or seeds, the former appear more species-rich. This is in line with results by Eriksson and Bremer (1991), and Bolmgren and Eriksson (2005), suggesting that there is a correlation between the evolution of fleshy fruits and an increased species diversity in woody plants.

Seed number reductions have also occurred in many independent clades of the Ixoroideae (12-14 transitions from numerous to few seeds per carpel, 1-2 transitions from few to one, and 8-9 transitions from numerous to one), none of which show a secondary increase in seed number. This is in support of Stebbins (1974) hypothesis of “adaptive modification along the lines of least resistance”, according to which reductions in the number of ovules tend to become fixed. Finally, we also provide diagnoses for the tribes Airospermeae (comprising *Airosperma* and *Boholia*), Augusteae (comprising *Augusta* and *Wendlandia*), and the monogeneric Scyphiphoreae, Steenisieae, and Trailliaedoxeae, as these genera represent clades that are not associated with any previously described tribes of the Ixoroideae.

Svensk sammanfattning

Systematik är en del av evolutionsbiologin och omfattar både förståelsen av olika organismgruppers evolutionära historik (fylogenetik) och deras indelning/klassifikation (taxonomi). Den grupp som här behandlas ingår i familjen Rubiaceae (kaffeväxter, krappväxter, eller mår(e)växter på svenska). Familjen är bland de mer artrika av blomväxterna, och är framförallt knuten till tropikerna med relativt få representanter i tempererade områden. Tidigare släktskapsstudier har visat att familjen kan betraktas som i huvudsak bestående av tre stora underfamiljer. Syftet med detta doktorandprojekt har varit att vidare undersöka släktskapsförhållanden inom en av dessa; Ixoroideae, främst med fokus på hur olika släkten, och grupper av släkten (tribus) kan vara släkt med varandra, samt hur dessa lämpligast indelas/klassificeras.

I de ingående artiklarna så har släktträd (fylogener) konstruerats utifrån jämförande analyser av olika regioner av växternas DNA. Sådana genträdd skall ses som hypoteser för gruppens evolutionära historia och stämmer inte nödvändigtvis överens med dess verkliga fylogeni. Så kallade molekylära fylogenetiska studier är ett alternativ eller komplement till studier av växternas form (morfologi), och kan bland annat användas för att testa om stöd finns för en viss taxonomisk indelning av en grupp. Fylogenetiska hypoteser är också viktiga exempelvis för frågeställningar rörande karaktärsutveckling (hur en karaktär/egenskap kan antas ha förändrats inom en grupp, och huruvida olika egenskaper kan antas vara korrelerade), biogeografi (varför gruppens utbredningsmönster ser ut som det gör), samt hybridisering.

Även om en (naturlig) indelning som återspeglar den evolutionära historien ofta har eftersträvat, så har växtklassifikation historiskt varit praktisk snarare än fylogenetisk. Grupper har karaktäriserats utifrån en bestämningsnyckel baserad på ett fåtal morfologiska karaktärer, vilket medför att grupperingarna i hög grad blir beroende av valet av nyckelkaraktärer. Vikten av en naturlig klassifikation har allt mer kommit att betonas, och numer anses bara fylogenetisk taxonomi som meningsfull. Naturliga (monofyletiska) grupper definieras av karaktärer (synapomorfier) som kan antas härstamma ifrån en gemensam anfader. En synapomorfi kan

ses som en karaktärsförändring från ett ursprungligt (plesiomorft) tillstånd. Genom att på så vis analysera karaktärsvariationen (vare sig den är av molekylärt, morfologiskt eller av annat slag) inom en grupp, så kan man vid tillräcklig variation (antal informativa karaktärer) teoretiskt rekonstruera dess fylogeni. Fylogenetisk rekonstruktion kompliceras av att karaktärer kan återgå till det ursprungliga tillståndet, och att analoga förändringar kan uppstå parallellt i olika klader (utvecklingslinjer/grenar). Därför är det viktigt att beakta ett större antal karaktärer.

Molekylära släktskapsstudier ger stöd för många traditionellt antagna växtgrupper, medan stora skillnader mot det förmodade släktskapet har påvisats inom andra. Fylogenetiska hypoteser baserade främst på kloroplast DNA har inneburit taxonomiska förändringar inom många grupper av kaffeväxter, och sammansättningen av de flesta tribus samt avgränsningarna av framförallt underfamiljerna Cinchonoideae och Ixoroideae har omvärderats.

Ixoroideae definierades ursprungligen som omfattande de släkten vars blommor har sekundär pollenpresentation (pollen avsatt och exponerat på pistillens stift), och ofta med kronblad vridna i knopp. Olika forskare har prioriterat dessa karaktärer olika. Molekylära fylogenetiska analyser stöder i huvudsak en sådan grupp, men har även indikerat att ett antal grupper som tidigare förts till underfamiljen Cinchonoideae verkar stå närmare Ixoroideae. Släktskapet mellan dessa grupper och med Ixoroideae (i mer traditionell bemärkelse) har varit det övergripande målet med detta projekt.

Speciellt fokus har givits två grupper som i tidigare studier verkat problematiska. En av dessa är tribus Alberteae, vars fylogenetiska position har varierat. Flera släkten har föreslagits vara närbesläktade (*Airosperma*, *Aulacocalyx*, *Boholia* och *Crossopteryx*). I den fylogenetiska hypotes som presenteras i artikel I, så ingår Alberteae i Coffeae-alliansen (se Figur 1), och inget av ovannämnda släkten står nära Alberteae. Vidare så är inte släktet *Alberta* monofyletiskt, då de inkluderade arterna ifrån Madagaskar står närmare släktet *Nematostylis*. Vi föreslår därför att dessa arter skall utgöra ett eget släkte; *Razafimandimbisonia*, och att Alberteae endast ska omfatta dessa tre släkten.

En annan problematisk grupp är Condamineae. Tidigare molekylära fylogenetiska studier har visat att Condamineae omfattar många släkten traditionellt placerade i olika tribus inom Cinchonoideae, samt ett antal släkten av osäker taxonomisk placering. Denna tidigare så olikartade indelning beror på en stor morfologisk variation inom gruppen. Släktskap och morfologisk karaktärsevolution inom denna grupp undersöks i artikel II. De analyserade karaktärerna (flera av vilka tidigare klassifikationer varit uppbyggda kring) såsom köttiga frukter, frövingar samt olika typer av

knopptäckning har sannolikt uppstått på flera håll inom gruppen. Fyra släkten (*Bathysa*, *Calycophyllum*, *Elaeagia* och *Rustia*) verkar inte vara monofyletiska med nuvarande släktomfattning, och vi föreslår därför att namnen *Holtonia*, *Schizocalyx* och *Semaphyllanthus* åter tas i bruk, att *Phitopsis* betraktas som synonymt med *Schizocalyx*, och *Tresanthera* som synonymt med *Rustia*.

I artikel III undersöks de fylogenetiska positionerna hos *Glionnetia*, *Jackiopsis* och *Trailliaedoxa*. *Glionnetia* har tidigare räknats till Rondeletieae (Cinchonoideae), medan de morfologiskt något säregna *Jackiopsis* och *Trailliaedoxa* inte placerats i senare klassifikationssystem. Liksom *Crossopteryx* (se artikel I) och *Scyphiphora*, så verkar även dessa släkten tillhöra Vanguerieae-alliansen, inom vilken de alla fem representerar utvecklingslinjer som har separerat relativt tidigt ifrån den utvecklingslinje som nu representeras av krongruppen (Aleisanthieae-Greeneae-Ixoreae-Vanguerieae). Jämfört med den senare är de alla artfattiga (monotypiska, med endast en art) och kan antas vara relikter. Vi föreslår därför att dessa släkten från ett perspektiv av biodiversitetsbevarande bör ges hög prioritet, detta utöver det faktum att de (med undantag för *Crossopteryx*) har liten utbredning och/eller växer i hotade ekosystem.

Slutligen, i artikel IV, så undersöks den morfologiska karaktärsutvecklingen inom Ixoroideae med fokus på de karaktärer som tidigare har ansetts viktiga i klassifikationen av Rubiaceae. Sekundär pollenpresentation vilket var den karaktär som ursprungligen användes för att definiera Ixoroideae representerar sannolikt en synapomorfi för den klad som utgörs av Coffeae-alliansen, Vanguerieae-alliansen och släktet *Retiniphyllum*. Vriden knopptäckning utgör sannolikt en synapomorfi för samma grupp samt *Steenisia*, det vill säga det är en karaktär som har uppstått tidigare. Båda karaktärerna har förlorats sekundärt inom vissa klader, och den senare har även uppstått parallellt inom andra grupper av Ixoroider. Mångfröiga kapslar verkar vara den ursprungliga frukttypen inom underfamiljen, från vilken många olika sorters bär, stenfrukter, och andra typer av frukter har uppstått inom olika klader. Inom ingen av dessa verkar en återgång till kapslar ha ägt rum. Frukttyp verkar även vara korrelerat med artantal, då de grupper vars frön sprids av djur är mer artrika än de (av samma ålder) med passivt spridda frukter eller frön. Även reduceringar i fröantal till få- eller en-fröiga karpeller har skett parallellt inom många oberoende grupper, till synes utan återgång till mångfröighet. I artikeln föreslår vi även att fem klader, inklusive de bestående av *Airosperma* och *Boholia* (artikel I), och *Trailliaedoxa* (artikel III), vilka inte är närbesläktade med tidigare beskrivna tribus enligt våra resultat, skall ses som egna tribus (Airospermeae, Augusteae, Scyphiphoreae, Steenisieae och Trailliaedoxeae).

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