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# Chemotaxonomy of the Rubiaceae family based on leaf fatty acid composition

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**Abstract**

With 10,700 species distributed in 637 genera, the Rubiaceae family is one of the largest of the angiosperms. Since it was previously evidenced that the fatty acid composition of photosynthetic tissues can be a tool for chemotaxonomic studies, the fatty acid composition of leaves from 107 Rubiaceae species highly representative of the diversity of the family was determined. Principal component analysis allowed a clear-cut separation of Coffeae, Psychotrieae and Rubieae. The occurrence of $C_{16:3}$ fatty acid, a marker of the prokaryotic plastidial lipid biosynthetic pathway, concerned at least two branches: Theligoneae/Rubieae and Anthospermeae–Anthosperminae which appeared to be in close relationship. Additional experiments were carried out to ensure the correlation between the presence of $C_{16:3}$ fatty acid and the prokaryotic biosynthetic pathway.


*Keywords:* Rubiaceae; Chemotaxonomy; Leaf fatty acids; Galactolipids; All *cis*-$\Delta^{7,10,13}$-hexadecatrienoic acid; Anteiso 17:0 fatty acid; Multivariate analysis

## 1. Introduction

Rubiaceae are mainly tropical woody plants and consist mostly of trees and shrubs, less often of perennial to annual herbs, as in Rubieae (subfamily Rubioideae) which are found in temperate regions. The Rubiaceae family is monophyletic and belongs to the Gentianales, as shown by cladistic analyses (Bremer, 1996). The most recent and complete classification (Robbrecht, 1988, 1993b) subdivided this large family into four subfamilies, namely Cinchonoideae, Ixoroideae, Antirheoideae and Rubioideae, corresponding to about 50 tribes. Nevertheless, molecular, morphological and chemical evidence to support the existence of the subfamily Antirheoideae is scanty (Bremer, 1996; Young et al., 1996; Rova et al., 1997, 2002; Andersson and Rova, 1999; etc.), and complex tribal revisions have been proposed (Andersson and Rova, 1999; Andreasen and Bremer, 2000; Bremer and Manen, 2000; Rova et al., 2002; etc.). For example, the tribe Isertieae of Robbrecht has become subdivided into three subfamilies; *Isertia* belonging to Cinchonoideae, *Mussaenda*/*Sabicea* belonging to Ixoroideae and *Mycetia* to Rubioideae.

The chemotaxonomic and phylogenetic relationships between angiosperms have already been investigated by studying the leaf fatty acid composition of 468 species (Mongrand et al., 1998). That study clearly showed that

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the fatty acid composition of photosynthetic tissues represents an interesting tool for chemotaxonomic studies of the angiosperm families, but also as more recently shown for the classification of gymnosperm species (Mongrand et al., 2001).

Regardless of their fatty acid composition, angiosperms can be divided into the so-called "16:3-plants" (those containing $C_{16:3}$ i.e. *all cis*-$\Delta^{7,10,13}$-hexadecatrienoic acid) and the "18:3-plants" which contain little, if any, $C_{16:3}$ (Heinz and Roughan, 1983; Mongrand et al., 1998; Moreau et al., 1998). Differences between 16:3-plants and 18:3-plants are reflected in variations in their plastidial lipid metabolism (Heinz and Roughan, 1983). Briefly, galactolipids, the major chloroplastic lipids, can be synthesized by two discrete pathways. The first (named the "prokaryotic pathway" because it also is found in cyanobacteria) is entirely located in the chloroplasts and leads to the synthesis of galactolipids containing tri-unsaturated C16 fatty acids. The second pathway (called the "eukaryotic pathway") results from the close cooperation between endoplasmic reticulum and chloroplasts (for review Browse and Somerville, 1991; Mongrand et al., 2000), and leads to the formation of plastid glycerolipids containing tri-unsaturated C18 fatty acids. In "16:3-plants", both pathways contribute to the synthesis of plastid lipids, so large amounts of $C_{16:3}$ and $C_{18:3}$ fatty acids are detected in their photosynthetic tissues. In "18:3-plants", the eukaryotic pathway is the only operative one, so little or no $C_{16:3}$ is observed. Indeed, chloroplasts from 16:3-plants possess the full equipment for the synthesis of plastid lipids (Heinz and Roughan, 1983), whereas plastids from 18:3-plants may be regarded as organelles which have lost some of their prokaryotic features. It follows that besides its chemometric interest, the $C_{16:3}/C_{18:3}$ balance could be a tool to study the phylogenetic relationships between plant species (Mongrand, 1998; Mongrand et al., 1998). From the latter studies, it became clear that in most cases, a given family among the 141 angiosperm families analyzed contains exclusively 16:3- or 18:3-plants. There are a few exceptions to this rule such as Hydrocharitaceae, Zosteraceae, and Rubiaceae because they contain both 16:3- and 18:3-plant species (Mongrand, 1998).

Although Rubiaceae family is one of the largest of the angiosperm families, the fatty acid composition of their leaves is poorly understood. The subfamily Cinchonoideae has remained totally unexplored to date and only six species belonging to Rubieae and Ixoroideae have been analyzed (Jamieson and Reid, 1971; Mongrand et al., 1998).

The present study sought to establish new phytochemical data on the Rubiaceae family, to begin to delimit the various subfamilies and tribes, and to disclose possible evolutionary tendencies among them. Hence, the leaf fatty acid composition of 107 Rubiaceae species was determined and was analyzed statistically. Additional experiments, i.e. in vivo [$^{14}$C]acetate short pulse experiments, were carried out to determine whether the presence of $C_{16:3}$ in some Rubiaceae specie is correlated with the existence of an intrachloroplastic prokaryotic biosynthesis of galactolipids.

## 2. Results

### 2.1. Overall leaf fatty acid composition of Rubiaceae

The overall leaf fatty acid composition of 107 Rubiaceae was determined by gas chromatography. Results presented in Table 1 are expressed as percent of total leaf fatty acids. The tribal classification is according to Robbrecht (1993b) and the intrafamilial classification according to Bremer (1996). Not surprisingly, in most species, linolenic (C18:3), linoleic (C18:2) and palmitic (C16:0) acids were the major fatty acids. However, some species showed an unexpected gas chromatographic pattern: no medium-chain fatty acids were detected in *Nertera depressa* leaves (no. 75); *Alberta magna* (no. 30) contained 8.9% 16:1Δ9-*cis*, 4.6% 16:1Δ3-*trans* and 12.4% of an unknown lipid compound eluting before 16:0; *Anthospermum rigidum* (no. 71) contained 15.8% of the latter compound; *Antirhea borbonica* (no. 6) was rich in 16:2, *Mussaenda tristigmatica* (no. 15) was rich in 18:0, and *Pavetta nitida*, *Tarenna supra-axillaris* and *Coffea myrtifolia* (no. 32, 34 and 45, respectively) were particularly rich in an undetermined lipid compound (probably a very long-chain fatty acid or very long-chain fatty alcohol). Among the Rubieae, *Galium lucidum* (no. 94) was remarkable for its higher concentrations of 18:0 and 18:1Δ9. Tribes confined to the subfamily Cinchonoideae by Bremer (1996) were rather rich in 18:2Δ9,12. This fatty acid reached 51.0% in *Hoffmannia refulgens* (no. 13), an Hamelieae. The genus *Coffea* appeared rich in 18:0 and 20:0, the tribe Psychotrieae had high concentrations of 18:1Δ9 and the tribe Rubieae had non-negligible concentrations of 16:1Δ3-*trans* and 16:3$\Delta^{7,10,13}$.

A statistical analysis was performed to separate the different tribes within the family by using only the overall leaf fatty acid compositions determined in this study. The best results were obtained by principal component analysis (PCA) with non-normalized and transformed values, the first and third principal components showing a certain distinction (Fig. 1). The discriminant variables were C18:2 (left) and C20:0 (right) for axis 1 and C18:1 (above) and 16:0 (down) for axis 3. Points right down had a high C18:0 value and points left down had a high 18:2 value. Ward hierarchical clustering did not allow a clear-cut distinction to be made between the three subfamilies (data not shown). The tribes Coffeae, Psychotrieae and Rubieae appeared to be separated by the PCA. *Theligonum cynocrambe* (no. 85) was found in

Table 1
Overall fatty acid composition of Rubiaceae leaf lipids

| Rubiaceae[a] | | (a) | 14:0 | (b) | 16:0 | 16:1Δ9-*cis* | 16:1Δ3-*trans* | 16:2Δ7,10 | 16:3Δ7,10,13 | 18:0 | 18:1Δ9 | 18:2Δ9,12 | 18:3Δ9,12,15 | 20:0 | (c) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cinchonoideae | | | | | | | | | | | | | | | |
| Coptosapelteae | | | | | | | | | | | | | | | |
| *Hymenodictyon floribundum* Robinson | 2 | 1 | 1.5 | 1.3 | 20.0 | 0.5 | 1.1 | 0.7 | 1.3 | 2.2 | 3.1 | 10.9 | 55.6 | 1.7 | 0.2 |
| *Mitragyna africana* Korth. | 3 | 3 | – | 1.2 | 20.6 | – | 1.5 | – | 1.2 | 2.6 | 0.2 | 17.4 | 55.3 | – | – |
| Naucleeae | | | | | | | | | | | | | | | |
| *Nauclea latifolia* Sm. | 4 | 1 | 0.6 | 0.8 | 19.2 | – | 1.2 | 0.5 | 1.0 | 4.1 | 5.6 | 19.4 | 46.0 | 1.8 | – |
| Rondeletieae | | | | | | | | | | | | | | | |
| *Rondeletia odorata* Jacq. | 5 | 3 | 1.9 | 2.1 | 30.2 | 0.6 | 1.4 | 0.5 | 1.3 | 2.5 | 6.1 | 28.4 | 25.0 | – | – |
| Guettardeae | | | | | | | | | | | | | | | |
| *Antirhea borbonica* J. F. Gmel. | 6 | 2 | 1.1 | 1.0 | 19.0 | – | 1.2 | 7.5 | 1.3 | 1.9 | 4.6 | 20.5 | 36.8 | 5.1 | – |
| *Guettarda speciosa* L. | 7 | 2 | 2.7 | 1.7 | 29.4 | – | 2.0 | 0.8 | 1.7 | 5.2 | 5.3 | 15.3 | 27.2 | 8.5 | – |
| *Guettarda uruquensis* Cham. & Schlecht. | 8 | 1 | 1.6 | 1.4 | 14.5 | – | 0.8 | – | 2.0 | 5.0 | 1.9 | 18.2 | 53.2 | 1.4 | – |
| Cephalantheae | | | | | | | | | | | | | | | |
| *Cephalanthus occidentalis* L. | 9 | 2 | 3.0 | 0.8 | 29.2 | 0.2 | 3.3 | 0.2 | 1.1 | 3.2 | 2.8 | 9.2 | 43.9 | 3.1 | – |
| Genera associated with *Portlandia* | | | | | | | | | | | | | | | |
| *Exostema longiflorum* (Lamb.) Roem. & Schult. | 10 | 3 | 1.3 | 1.3 | 22.6 | – | – | – | 1.1 | 3.7 | 4.4 | 21.4 | 44.1 | – | – |
| Chiococceae | | | | | | | | | | | | | | | |
| *Chiococca alba* (L.) Hitchc. | 11 | 3 | 2.1 | 1.2 | 27.1 | 1.0 | 0.8 | – | 1.3 | 2.4 | 6.0 | 17.3 | 38.7 | 2.1 | – |
| Hamelieae | | | | | | | | | | | | | | | |
| *Hamelia patens* Jacq. | 12 | 2 | 1.7 | 1.8 | 26.5 | – | 1.2 | – | 1.9 | 3.4 | 6.5 | 17.8 | 37.4 | 1.9 | – |
| *Hoffmannia refulgens* Hemsl. | 13 | 2 | 2.2 | 2.0 | 16.8 | 0.7 | 1.7 | 0.3 | 1.6 | 0.7 | 2.3 | 51.0 | 20.0 | 0.7 | – |
| Ixoroideae | | | | | | | | | | | | | | | |
| Isertieae | | | | | | | | | | | | | | | |
| *Mussaenda sanderiana* Ridley | 14 | 2 | – | 1.2 | 46.9 | – | 1.7 | – | 2.2 | 4.8 | 6.6 | 10.8 | 23.1 | 2.8 | – |
| *Mussaenda tristigmatica* Cummins | 15 | 1 | 0.4 | 0.8 | 27.5 | – | 0.3 | 0.3 | 0.9 | 23.9 | 2.9 | 9.4 | 29.8 | 3.8 | – |
| *Mycetia longifolia* K. Schum. | 1 | 2 | 1.4 | 1.4 | 23.0 | 0.5 | 0.7 | 0.5 | 1.6 | 2.1 | 5.7 | 16.8 | 45.4 | 1.0 | – |
| *Sabicea calycina* Benth. | 16 | 2 | 2.3 | 2.1 | 30.8 | 0.9 | 2.6 | 0.6 | 2.2 | 6.6 | 4.3 | 9.8 | 35.6 | 2.1 | tr. |
| Gardenieae | | | | | | | | | | | | | | | |
| *Gardenia cornuta* Hemsl. | 17 | 1 | 1.9 | 0.6 | 19.5 | – | 0.6 | 0.8 | 0.8 | 4.7 | 6.6 | 18.6 | 43.1 | 2.2 | 0.8 |
| *Gardenia spatulifolia* Stapf & Hutch. | 18 | 1 | 1.2 | 0.7 | 22.3 | – | 0.8 | 0.5 | 0.8 | 4.0 | 6.0 | 6.4 | 55.8 | 1.5 | – |
| *Gardenia taitensis* DC. | 19 | 2 | 2.7 | 1.0 | 20.9 | – | 0.6 | 0.6 | 0.9 | 2.7 | 6.0 | 12.0 | 48.1 | 2.2 | 2.2 |
| *Gardenia thunbergia* L. f. | 20 | 1 | 1.6 | 1.0 | 17.6 | – | 1.1 | 0.5 | 1.4 | 4.0 | 3.3 | 8.0 | 59.9 | 1.6 | – |

(*continued on next page*)

Table 1 (*continued*)

| Rubiaceae[a] | | (a) | 14:0 | (b) | 16:0 | 16:1Δ9-*cis* | 16:1Δ3-*trans* | 16:2Δ7,10 | 16:3Δ7,10,13 | 18:0 | 18:1Δ9 | 18:2Δ9,12 | 18:3Δ9,12,15 | 20:0 | (c) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Genipa americana* L. | 21 | 2 | 1.5 | 0.6 | 18.8 | 0.2 | 1.5 | 0.4 | 0.7 | 3.9 | 2.7 | 27.4 | 40.9 | 1.4 | – |
| *Macrosphyra longistyla* Hook. f. | 22 | 1 | 2.3 | 0.7 | 22.7 | – | 1.0 | 0.8 | 0.9 | 4.0 | 7.6 | 10.0 | 45.8 | 1.3 | 2.8 |
| *Oxyanthus formosus* Hook. f. | 23 | 1 | 2.6 | 0.9 | 22.6 | – | 1.2 | 0.9 | 1.0 | 4.8 | 1.8 | 7.0 | 55.6 | 1.5 | – |
| *Randia dumetorum* Lam. | 24 | 2 | 1.9 | 1.1 | 19.5 | 0.4 | 1.3 | 0.7 | 1.6 | 5.3 | 7.5 | 7.2 | 49.9 | 2.1 | 1.5 |
| *Randia formosa* (Jacq.) K. Schum. | 25 | 2 | 1.8 | 1.1 | 23.6 | 0.5 | 2.1 | 0.5 | 1.4 | 5.3 | 3.5 | 10.0 | 47.7 | 2.4 | – |
| *Rothmannia annae* (E. P. Wright) Keay | 26 | 2 | 2.0 | 0.8 | 22.9 | 0.4 | 1.8 | 0.7 | 0.8 | 2.5 | 3.4 | 11.1 | 50.7 | 1.4 | 1.4 |
| *Cremaspora triflora* (Thonn.) K. Schum. | 27 | 3 | 2.9 | 1.8 | 23.5 | – | – | 0.6 | 1.9 | 7.1 | 7.8 | 7.0 | 47.3 | – | – |
| Vanguerieae | | | | | | | | | | | | | | | |
| *Psydrax odorata* (Forst. f.) A. C. Smith & S. P. Darwin | 28 | 2 | 1.6 | 0.9 | 21.7 | 0.4 | tr. | 0.3 | 0.8 | 3.4 | 6.5 | 15.6 | 47.4 | 1.0 | – |
| *Vangueria madagascariensis* J. F. Gmel. | 29 | 1 | 1.9 | 1.4 | 20.9 | 1.0 | 1.7 | 0.5 | 1.3 | 9.4 | 3.8 | 12.3 | 43.7 | 2.2 | – |
| Alberteae | | | | | | | | | | | | | | | |
| *Alberta magna* E. Mey. | 30 | 3 | 1.4 | 12.4 | 17.5 | 8.9 | 4.6 | 1.0 | 1.1 | 2.1 | 2.3 | 7.8 | 39.8 | 1.1 | – |
| Pavetteae | | | | | | | | | | | | | | | |
| *Myonima obovata* Lam. var. *obovata* | 31 | 2 | 2.8 | 0.8 | 20.0 | – | 0.4 | 0.9 | 0.9 | 3.1 | 4.2 | 14.2 | 50.2 | 2.5 | – |
| *Pavetta nitida* (Schum. & Thonn.) Hutch. & Dalz. | 32 | 1 | 1.2 | 1.4 | 14.3 | 0.2 | 0.4 | 0.3 | 1.0 | 4.0 | 2.9 | 8.8 | 35.3 | 1.1 | 29.1 |
| *Tarenna borbonica* (E. G. & A. Henders.) Verdc. | 33 | 2 | 1.9 | 1.7 | 18.6 | 0.4 | 0.7 | 0.3 | 1.4 | 2.9 | 6.1 | 5.7 | 57.5 | 0.8 | 2.0 |
| *Tarenna supra-axillaris* (Hemsl.) Bremek. | 34 | 2 | 0.4 | 1.1 | 21.7 | 0.7 | 1.2 | 0.3 | 1.1 | 2.4 | 9.2 | 11.9 | 32.7 | 1.2 | 16.2 |
| *Tarenna trichantha* (Baker) Bremek. | 35 | 2 | 1.7 | 1.4 | 20.7 | 0.5 | 1.8 | 0.3 | 1.6 | 4.1 | 7.3 | 9.4 | 48.8 | 1.6 | 0.8 |
| *Tarenna verdcourtiana* Fosberg | 36 | 2 | 1.8 | 1.7 | 23.1 | 0.5 | 1.3 | 0.4 | 1.8 | 3.2 | 15.9 | 11.9 | 36.2 | 1.4 | 0.8 |
| Coffeeae | | | | | | | | | | | | | | | |
| *Coffea arabica* L. | 37 | 4 | 2.5 | 0.9 | 23.5 | 0.6 | 1.9 | 0.5 | 0.7 | 7.5 | 1.9 | 1.0 | 57.0 | 2.1 | – |
| *Coffea boiviniana* (Baill.) A. Cheval. | 38 | 1 | 1.7 | 1.3 | 25.0 | – | 1.3 | 0.9 | 2.0 | 8.7 | 4.8 | 16.6 | 34.1 | 3.6 | – |
| *Coffea canephora* Pierre ex Froehn. | 39 | 1 | 2.7 | 0.7 | 18.4 | – | 0.9 | 1.4 | 0.8 | 11.8 | 1.7 | 8.5 | 50.5 | 2.5 | – |
| *Coffea ebracteolata* (Hiern) Brenan | 40 | 1 | 3.1 | 0.7 | 35.6 | – | 0.5 | 1.0 | 0.9 | 7.6 | 3.3 | 4.5 | 40.3 | 2.3 | – |
| *Coffea excelsa* Cheval. | 41 | 1 | 1.7 | 0.8 | 26.9 | – | 0.7 | 1.0 | 1.1 | 15.6 | 8.8 | 5.1 | 36.7 | 1.7 | – |
| *Coffea laurina* Smeathm. ex DC. | 42 | 1 | 3.3 | 3.1 | 16.4 | – | 0.8 | 1.3 | 1.0 | 12.6 | 2.3 | 4.3 | 52.9 | 2.1 | – |
| *Coffea liberica* Bull. ex Hiern | 43 | 1 | 1.8 | 0.7 | 19.0 | – | 0.9 | 0.7 | 0.7 | 8.3 | 2.7 | 16.5 | 46.7 | 1.9 | – |
| *Coffea mauritiana* Lam. | 44 | 1 | 3.9 | 0.8 | 24.4 | 0.6 | 1.0 | 0.9 | 0.9 | 8.0 | 5.9 | 11.9 | 39.5 | 1.7 | 0.3 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Coffea myrtifolia* (A. Rich. ex DC.) J. F. Leroy | 45 | 1 | 2.8 | 1.2 | 33.2 | 1.8 | 2.1 | 0.5 | 1.0 | 10.5 | 11.6 | 2.5 | 6.2 | 2.5 | 24.2 |
| *Coffea racemosa* Lour. | 46 | 2 | 2.2 | 1.0 | 30.0 | 1.0 | 1.7 | 0.7 | 1.0 | 7.2 | 6.6 | 15.2 | 31.3 | 2.0 | – |
| *Coffea rupestris* Hiern | 47 | 1 | 3.1 | 0.8 | 13.8 | – | 1.2 | 1.0 | 0.7 | 13.0 | 2.2 | 5.6 | 55.1 | 3.6 | – |
| *Coffea vaughanii* J. F. Leroy | 48 | 1 | 1.3 | 0.5 | 31.6 | 0.6 | 2.0 | 0.4 | 0.2 | 9.8 | 13.7 | 6.7 | 29.9 | 2.8 | 0.3 |
| *Coffea zanguebariae* Lour. | 49 | 1 | 1.2 | 1.0 | 25.8 | 0.6 | 1.5 | 0.4 | 1.0 | 7.9 | 3.7 | 13.8 | 40.2 | 2.2 | 0.5 |
| *Psilanthus melanocarpus* (Welw. ex Hiern) J. F. Leroy | 50 | 1 | 1.4 | 1.3 | 19.7 | – | 1.1 | 0.5 | 1.7 | 3.8 | 3.8 | 6.4 | 56.8 | 2.3 | 1.1 |
| Octotropidae | | | | | | | | | | | | | | | |
| *Fernelia buxifolia* Lam. | 51 | 2 | 1.3 | 1.5 | 23.2 | 0.9 | 1.0 | 1.0 | 1.4 | 2.8 | 5.3 | 18.9 | 40.0 | 2.7 | – |
| Rubioideae | | | | | | | | | | | | | | | |
| Ophiorrhizeae | | | | | | | | | | | | | | | |
| *Ophiorrhiza mungos* L. | 52 | 1 | 1.6 | 1.5 | 24.9 | 1.0 | 1.8 | 0.3 | 1.7 | 3.1 | 9.8 | 19.0 | 33.6 | 1.8 | – |
| Coccocypseleae | | | | | | | | | | | | | | | |
| *Coccocypselum guianense* (Aubl.) K. Schum. | 70 | 3 | 1.4 | 1.1 | 21.3 | – | 1.2 | – | 1.0 | 2.8 | 4.3 | 21.7 | 45.1 | – | – |
| Psychotrieae | | | | | | | | | | | | | | | |
| *Chassalia corallioides* (Cordem.) Verdc. | 53 | 2 | 1.2 | 1.2 | 19.4 | 1.2 | 1.4 | 0.6 | 1.5 | 2.9 | 14.3 | 8.2 | 46.5 | 1.7 | – |
| *Geophila repens* (L.) Johnston | 54 | 2 | 1.7 | 1.3 | 19.9 | 2.0 | 0.9 | 0.8 | 1.7 | 1.2 | 12.5 | 16.4 | 39.9 | 1.6 | – |
| *Psathura borbonica* J. F. Gmel. var. *borbonica* | 55 | 2 | 1.6 | 1.2 | 19.0 | 0.3 | 1.2 | 0.7 | 1.2 | 2.5 | 10.7 | 12.1 | 48.2 | 1.2 | – |
| *Psychotria bacteriophila* G. Valet. | 56 | 2 | 1.0 | 0.9 | 18.2 | 0.8 | 1.4 | 0.3 | 0.9 | 1.8 | 16.3 | 9.9 | 47.3 | 1.1 | – |
| *Psychotria carthagenensis* Jacq. | 57 | 1 | 2.3 | 1.1 | 21.3 | – | 1.7 | 0.7 | 1.3 | 2.5 | 7.2 | 7.1 | 46.6 | 8.2 | – |
| *Psychotria guadalupesis* (DC.) Howard | 58 | 2 | 1.5 | 1.7 | 21.9 | 0.4 | 1.4 | 0.5 | 0.8 | 3.4 | 9.8 | 13.1 | 42.1 | 2.3 | 1.3 |
| *Psychotria hirtella* Oliver | 59 | 2 | 1.1 | 1.0 | 20.8 | 0.6 | 1.3 | 0.4 | 1.0 | 2.0 | 17.4 | 9.2 | 43.6 | 1.3 | tr. |
| *Psychotria nervosa* Sw. | 60 | 1 | 1.3 | 1.4 | 18.3 | – | 2.0 | 0.5 | 1.9 | 1.6 | 10.5 | 7.9 | 53.9 | 0.8 | – |
| *Psychotria sodifera* De Wild. | 61 | 1 | 1.3 | 1.4 | 21.2 | – | 1.5 | 0.4 | 1.2 | 2.8 | 16.9 | 5.6 | 43.5 | 4.4 | – |
| *Psychotria viridiflora* Reinw. ex Blume | 62 | 1 | 2.6 | 1.1 | 21.8 | – | 2.2 | – | 1.1 | 2.1 | 10.7 | 8.4 | 37.8 | 12.2 | – |
| *Hydnophytum formicarum* Jack | 63 | 2 | 1.4 | 1.0 | 22.8 | 1.6 | – | 0.8 | 0.7 | 5.1 | 20.5 | 9.3 | 32.6 | 4.2 | – |
| *Hydnophytum papuanum* Becc. | 64 | 2 | 3.7 | 0.9 | 25.8 | 1.4 | 1.3 | 0.6 | 0.8 | 3.5 | 13.3 | 7.8 | 37.4 | 3.1 | 0.4 |
| *Myrmecodia echinata* Miq. | 65 | 2 | 2.1 | 1.2 | 25.9 | – | 1.5 | 0.5 | 0.4 | 3.1 | 14.2 | 9.4 | 40.4 | 1.3 | – |
| *Myrmecodia platyrea* Becc. | 66 | 2 | 3.3 | 1.2 | 25.6 | – | 1.5 | 0.7 | 0.3 | 2.6 | 5.0 | 7.9 | 49.2 | 1.8 | 0.6 |
| *Myrmecodia tuberosa* Jack | 67 | 2 | 2.7 | 0.9 | 24.9 | 0.5 | 1.3 | 0.6 | 0.6 | 3.2 | 12.9 | 13.8 | 36.4 | 1.8 | 0.4 |
| *Myrmephytum selebicum* Becc. | 68 | 2 | 2.5 | 1.4 | 25.2 | 0.4 | 1.2 | 1.3 | 0.8 | 3.8 | 13.1 | 5.1 | 41.4 | 2.5 | 1.3 |
| Morindeae | | | | | | | | | | | | | | | |
| *Morinda citrifolia* L. (=*M. lucida* Benth.) | 69 | 3 | 1.6 | 0.8 | 23.9 | 0.2 | 0.8 | 0.2 | 0.9 | 2.8 | 5.6 | 9.1 | 51.8 | 2.4 | – |

(*continued on next page*)

Table 1 (*continued*)

| Rubiaceae[a] | | (a) | 14:0 | (b) | 16:0 | 16:1Δ9-*cis* | 16:1Δ3-*trans* | 16:2Δ7,10 | 16:3Δ7,10,13 | 18:0 | 18:1Δ9 | 18:2Δ9,12 | 18:3Δ9,12,15 | 20:0 | (c) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Anthospermeae | | | | | | | | | | | | | | | |
| *Anthospermum rigidum* Eckl. & Zeyh. | 71 | 3 | 2.2 | 15.8 | 18.5 | – | 1.5 | – | 3.9 | 1.3 | 2.1 | 13.8 | 41.0 | – | – |
| *Phyllis nobla* L. | 72 | 3 | 0.7 | 1.1 | 24.0 | – | 0.9 | 3.7 | 3.8 | 1.5 | 12.0 | 27.6 | 24.8 | – | – |
| *Coprosma baueri* Endl. | 73 | 4 | 1.8 | 1.2 | 29.3 | 1.0 | 1.2 | 0.9 | 2.1 | 2.4 | 8.9 | 12.7 | 36.7 | 1.7 | – |
| *Coprosma repens* Hook. f. | 74 | 4 | 1.3 | 1.3 | 22.3 | 0.4 | tr. | 1.0 | 1.1 | 2.8 | 11.1 | 11.3 | 45.2 | 1.0 | 1.0 |
| *Nertera depressa* Banks & Soland. ex Gaertn. | 75 | 4 | – | – | 21.0 | – | – | – | – | 2.8 | 5.9 | 26.2 | 44.1 | – | – |
| Spermacoceae | | | | | | | | | | | | | | | |
| *Mitracarpum lhotzkianum* Cham. | 76 | 3 | 2.8 | 1.2 | 20.1 | 0.6 | 1.3 | 0.8 | 0.6 | 1.5 | 3.7 | 15.2 | 52.2 | – | – |
| *Richardia scabra* L. | 77 | 4 | 2.6 | 0.9 | 20.4 | 0.5 | 1.2 | 0.2 | 0.9 | 2.6 | 6.4 | 13.9 | 49.2 | 1.3 | – |
| *Spermacoce verticillata* L. | 78 | 2 | 1.3 | 1.3 | 17.0 | – | 1.2 | – | 1.1 | 2.0 | 7.6 | 22.5 | 43.5 | 2.5 | – |
| Hedyotideae | | | | | | | | | | | | | | | |
| *Bouvardia leiantha* Benth. | 79 | 1 | 1.0 | 1.1 | 21.1 | – | 2.7 | – | 1.2 | 2.4 | 7.7 | 21.7 | 40.5 | – | 0.6 |
| *Manettia inflata* Sprague | 80 | 2 | 1.3 | 1.2 | 23.1 | 1.8 | 0.7 | – | 2.0 | 3.3 | 10.1 | 22.9 | 31.6 | 1.9 | – |
| *Pentas lanceolata* Schum. = *P. carnea* Benth. | 81 | 4 | 1.6 | 0.9 | 35.6 | 1.3 | 1.3 | 0.6 | 1.2 | 4.9 | 9.9 | 20.1 | 19.9 | 2.9 | – |
| Paederieae | | | | | | | | | | | | | | | |
| *Paederia scandens* (Lour.) Merrill | 82 | 1 | 1.2 | 0.8 | 19.2 | 0.5 | 1.7 | 0.2 | 1.1 | 0.9 | 6.4 | 15.7 | 51.1 | 1.0 | 0.3 |
| *Serissa fetida* Lam. | 83 | 6 | 0.9 | 0.9 | 19.0 | 0.2 | 0.9 | 0.3 | 1.0 | 1.9 | 5.4 | 18.5 | 49.6 | 1.0 | 0.2 |
| *Spermadictyon suaveolens* Roxb. | 84 | 1 | 1.1 | 1.2 | 20.2 | 0.5 | 0.7 | 0.7 | 1.4 | 1.7 | 5.1 | 14.6 | 52.2 | 0.6 | – |
| Theligoneae | | | | | | | | | | | | | | | |
| *Theligonum cynocrambe* L. | 85 | 4 | 0.9 | 1.0 | 17.7 | – | 2.5 | 0.7 | 8.0 | 0.9 | 8.6 | 15.4 | 44.3 | – | – |
| Rubieae | | | | | | | | | | | | | | | |
| *Asperula cynanchica* L. | 86 | 5 | 1.4 | 1.7 | 18.4 | 0.5 | 3.1 | 0.2 | 4.0 | 1.6 | 4.8 | 15.5 | 45.6 | 1.7 | 1.6 |
| *Asperula purpurea* (L.) Ehrend. | 87 | 5 | 0.7 | 2.0 | 17.8 | 1.7 | 3.5 | 1.0 | 3.0 | 2.6 | 7.7 | 19.0 | 41.0 | – | – |
| *Asperula tinctoria* L. | 88 | 5 | 1.5 | 0.2 | 22.2 | 0.8 | 2.1 | 0.9 | 4.4 | 1.6 | 3.8 | 11.7 | 47.2 | 3.6 | – |
| *Cruciata chersonensis* (Willd.) Ehrend. | 89 | 5 | 1.2 | 0.8 | 21.0 | 0.7 | 2.1 | 0.7 | 4.2 | 1.3 | 8.7 | 13.2 | 46.1 | – | – |
| *Galium arenarium* Loisel. | 90 | 7 | 1.8 | 0.7 | 23.5 | 0.6 | 1.7 | 0.5 | 2.8 | 1.5 | 4.9 | 19.8 | 35.2 | 2.5 | 4.5 |
| *Galium divaricatum* Pourret ex Lam. | 91 | 5 | 1.1 | 1.0 | 17.0 | 0.3 | 2.1 | 0.5 | 5.1 | 0.9 | 5.0 | 15.8 | 50.8 | 0.5 | – |
| *Galium glaucum* L. | 92 | 5 | 1.3 | 1.0 | 16.7 | 0.8 | 2.8 | 0.3 | 6.7 | 1.0 | 4.7 | 14.5 | 49.4 | 0.9 | – |
| *Galium hypocarpium* (L.) Endl. ex Griseb. | 93 | 3 | 1.5 | 1.7 | 21.2 | – | 1.9 | – | 8.3 | 1.5 | 1.7 | 11.1 | 51.2 | – | – |
| *Galium lucidum* All. | 94 | 5 | 4.0 | 3.1 | 27.9 | – | 0.8 | 0.8 | 2.3 | 14.9 | 11.1 | 9.3 | 25.7 | – | – |
| *Galium marchandii* Roemer & Schultes | 95 | 5 | 2.8 | tr. | 17.9 | 0.5 | 1.8 | 1.8 | 2.3 | 1.3 | 6.1 | 18.5 | 46.9 | – | – |
| *Galium mollugo* L. | 96 | 7 | 1.9 | 1.0 | 18.6 | 1.8 | 2.1 | 0.4 | 5.5 | 1.5 | 3.6 | 10.8 | 51.5 | 1.2 | – |
| *Galium palustre* L. | 97 | 5 | 0.7 | 1.0 | 16.4 | 0.4 | 1.8 | 0.4 | 3.4 | 1.7 | 5.1 | 23.7 | 40.1 | 2.1 | 3.2 |
| *Galium parisiense* L. | 98 | 5 | 1.2 | 0.7 | 17.4 | – | 1.8 | 0.2 | 3.5 | 2.6 | 5.9 | 14.8 | 48.2 | 3.6 | – |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Galium saxatile* L. | 99 | 5 | 1.2 | 1.4 | 16.4 | 0.8 | 2.8 | 0.4 | 5.9 | 0.7 | 5.5 | 14.6 | 50.1 | 0.2 | – |
| *Galium sylvaticum* L. | 100 | 5 | 2.0 | 0.2 | 20.1 | 1.6 | 2.8 | 0.5 | 3.3 | 2.1 | 5.9 | 14.3 | 43.3 | 3.8 | – |
| *Galium timeroyi* Jord. | 101 | 5 | 1.0 | 0.8 | 16.5 | 0.8 | 1.9 | 0.5 | 2.4 | 1.4 | 6.6 | 19.0 | 44.7 | 4.6 | – |
| *Galium verum* L. | 102 | 5 | 1.5 | 0.9 | 19.0 | 1.6 | 2.1 | 0.4 | 3.4 | 1.3 | 4.4 | 15.6 | 47.7 | 2.0 | – |
| *Galium capense* subsp. *Garipense* | 103 | 3 | 2.2 | – | 24.0 | – | – | – | 1.5 | 2.3 | 5.7 | 19.2 | 45.1 | – | – |
| *Phuopsis stylosa* Benth. et Hook. | 104 | 4 | 1.1 | 1.2 | 15.4 | 0.4 | 2.6 | 0.2 | 9.3 | 1.0 | 3.5 | 12.7 | 52.0 | 0.8 | – |
| *Rubia peregrina* L. | 105 | 7 | 2.8 | 1.5 | 26.6 | 0.8 | 2.7 | 0.4 | 2.9 | 1.9 | 6.8 | 16.9 | 36.6 | – | – |
| *Rubia tinctorum* L. | 106 | 5 | 0.2 | 0.4 | 16.7 | 0.3 | 2.5 | 0.8 | 7.1 | 0.7 | 4.2 | 18.5 | 45.8 | 0.9 | 1.8 |
| *Sherardia arvensis* L. | 107 | 7 | 2.4 | 0.8 | 19.6 | 0.7 | 1.8 | 0.4 | 6.6 | 1.8 | 2.9 | 9.3 | 52.1 | 1.6 | – |

Species are classified according to the tribes of Robbrecht (1993b) and distributed according to Bremer's work (1996). Values are expressed as percentages of total.
–: Not detected.
tr.: Trace, percentage less than 0.05%.
(a): Origin of Rubiaceae leaves: (1): Arboretum of Chèvreloup (France); (2): Botanical garden of Nancy (France) (3): National Botanic Garden of Belgium (Domain of Bouchout, Meise); (4) Botanical garden of Talence (France); (5): Botanical garden of Bordeaux (France); (6) Botanical garden of Iasi (Romania); (7) leaves sampled in nature near Bordeaux (France).
(b): Undetermined medium chain fatty acid eluting just before 16:0 acid but distinct from it (probably 15:0 or branched 15:0).
(c): Undetermined lipid compound.
[a] Tribes proposed by Robbrecht (1988, 1993b).

the vicinity of Rubieae. Within the Rubieae, *Galium capense* subsp. *garipense* (no. 103) had an eccentric position. The tribe Paederieae (no. 82–84) was grouped near Rubieae, and *Morinda citrifolia* (no. 69, a Morindeae) was not far from the Psychotrieae. Most of the Cinchonoideae subfamily and most of the Coccocypseleae, Anthospermeae (but not genus *Coprosma*), Spermacoceae, and Hedyotideae tribes occupied the low left side of the diagram. The Coffeeae were almost all located low on the right side of the diagram and the Pavetteae (no. 33, 35, 36) were grouped in the upper position.

As discussed in Section 1, because of its prokaryotic origin the $C_{16:3}$ fatty acid content was carefully analyzed. This fatty acid ranged from 2.4% to 9.3% of total leaf fatty acids (see Mongrand et al., 1998) in 22 species belonging to six different genera of Rubieae. All of the other members of Rubiaceae appeared as 18:3-plants. Thus, the Rubieae tribe appeared as a16:3-plant group. The sole exception in the Rubieae tribe concerns *Galium capense* subsp. *garipense* with only 1.5% $C_{16:3}$. *Theligonum cynocrambe* (monogeneric tribe of Theligoneae) also contained 8% $C_{16:3}$. In the Anthospermae tribe, the genera *Phyllis* and *Anthospermum* are likely to be 16:3-plants whereas *Coprosma* and *Nertera* are 18:3 plants.

### 2.2. In vivo pulse-labeling of some Rubiaceae leaf lipids

The analysis of leaf fatty acid composition of Rubiaceae evidenced two groups distinguished by the presence of $C_{16:3}$ (Table 1). To ensure that this fatty acid is well correlated with the presence of the plastidial prokaryotic pathway, we carried out a short pulse using sodium [1-$^{14}$C]acetate, a precursor for fatty acid synthesis.

In 16:3-plants, prokaryotic galactolipids (mainly MGDG) are synthesized in chloroplasts concomitantly with phosphatidylcholine (PC) in endomembranes. In such plants, after a short pulse, the ratio MGD/PC is close to 1 (Roughan and Slack, 1982). By contrast, prokaryotic galactolipid synthesis does not occur in 18:3 plants, and galactolipids are exclusively synthesized after an import of lipids previously synthesized in reticulum membranes (Roughan and Slack, 1982; Mongrand et al., 1998, 2000). Thus, after a short [$^{14}$C]acetate pulse, the ratio MGD/PC from 18:3 plants is significantly inferior to 1 (Roughan and Slack, 1982). Nevertheless, one can imagine an alternative plastidial lipid biosynthesis – not yet described in the literature – leading to an erroneous correlation between the fatty acid marker $C_{16:3}$and the prokaryotic pathway. Experiments were therefore performed by using fresh leaves from two Rubiaceae 18:3-plant species (*Coffea arabica* and *Pentas lanceolata*), and from one 16:3-plant species containing 9.3% of this fatty acid (*Phuopsis stylosa*). After the 30-min pulse, the ratio MGD/PC was 1.23 for *Phuopsis stylosa* and 0.18 for the other ones (Table 2). These re-

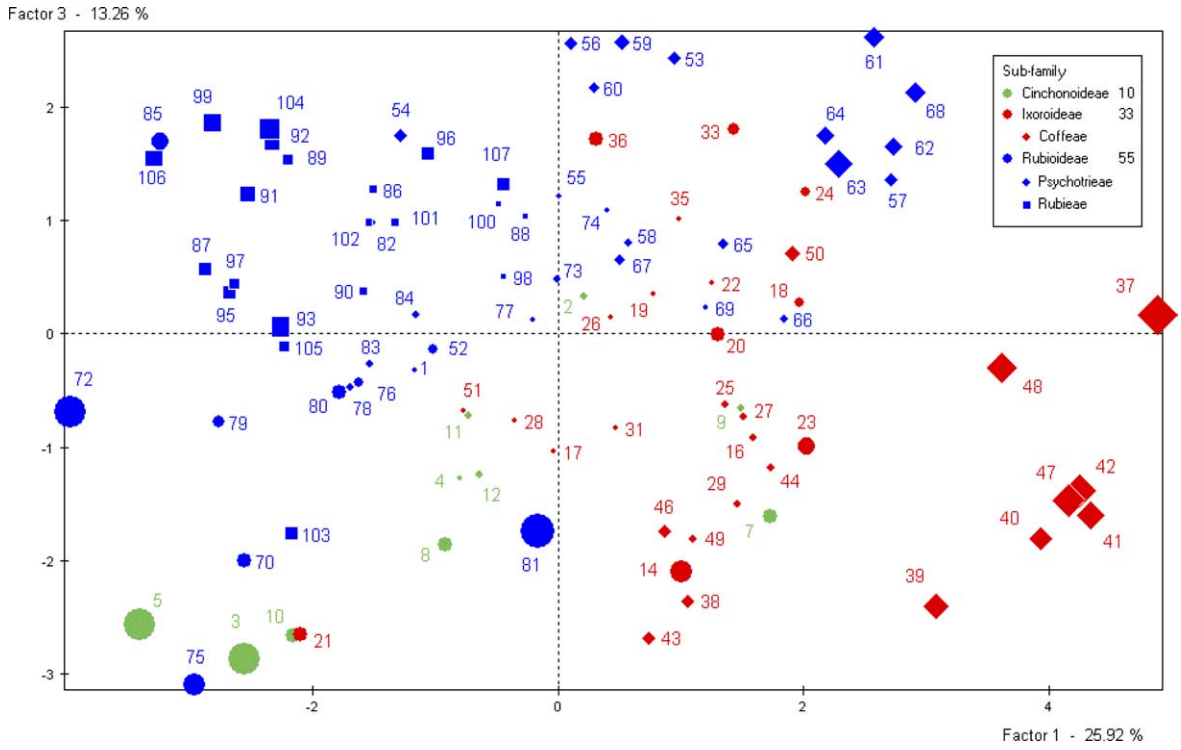


Fig. 1. Principal component analysis of 98 of the 107 Rubiaceae taxa, indexed from 1 to 107 according to Table 1 and following the intrafamilial classification of Bremer (1996). The size of each dot represents the quality of representation. Nine species were omitted during the calculation process because of their very peculiar profile.

Table 2
Na [$^{14}$C]acetate incorporation in glycerolipids of three Rubiaceae leaves

| | Radioactivity incoporated (% of total polar lipids) | | | | | Ratio MGDG/PC |
|---|---|---|---|---|---|---|
| | MGDG | DGDG | PC | PG +;PE | PI + PS | |
| *Phuopsis stylosa* Benth. et Hook. | 28.85 | 6.64 | 23.39 | 29.05 | 12.6 | 1.23 |
| *Coffea arabica* L. | 9.27 | 8.15 | 50.61 | 15.61 | 16.35 | 0.18 |
| *Pentas lanceolata* Schum. | 7.32 | 8.73 | 39.38 | 30.82 | 13.84 | 0.18 |

[1-$^{14}$C]Acetate (86 nCi) was supplied for 30 min on Rubiaceae leaves. Lipids were then extracted and purified by monodimensional HPTLC. The radioactivity associated with the lipids was determined using a PhosphorImager cassette and signals were quantified using ImageQuaNT software (Molecular Dynamic).

sults clearly suggest the presence of a plastidial galactolipid synthesis in *Phuopsis stylos* and its absence in *Coffea arabica* and *Pentas lanceolata*.

## 3. Discussion

This study of the leaf fatty acid composition of 107 Rubiaceae species shows that leaf fatty acid composition has a taxonomic significance at the tribal level. PCA allowed the separation of Coffeae, Psychotrieae and Rubieae, and brought Theligoneae close to Rubieae. At the subtribal level, Anthosperminae and Coprosminae, which are subtribes of Anthospermeae, are separated (see Fig. 1). Moreover, both 16:3- and 18:3-plants are part of the Rubiaceae family analyzed in this study. Rubieae, Theligoneae and some Anthospermae belong to the first group whereas the other Rubiaceae species are clustered in the second group.

According to sequence comparison data (*rbc*L, *atp*B*rbc*L, *rps*16 intron), the tribe Rubieae forms a homogeneous group and is clearly monophyletic (Manen et al., 1994; Manen and Natali, 1996; Bremer, 1996; Natali et al., 1996; Andersson and Rova, 1999; Bremer and Manen, 2000). Moreover, compared to the other Rubiaceae, the Rubieae species are predominantly herbaceous, but some woody representatives are thought to show secondary woodiness (Koek-Noorman, 1976; Robbrecht, 1988; Jansen et al., 2002). It is still not absolutely sure whether these woody species have originated from herbaceous ancestors, because the molecular trees are not sufficiently resolved so far. The derived state of their interpetiolar stipules transformed into a whorl of Rubieae leaves is met within no other Rubiaceae. Among

all the Rubieae analyzed in this study, *Galium capense* subsp. *garipense* is an exception since it contains very low amounts of $C_{16:3}$ fatty acid.

The data also show that *Theligonum* is a 16:3-plant. The Rubiaceous affinity of *Theligonum* was described belatedly. It is a plant that has numerous remarkable features: polyandry of male flowers, lack of calyx, flowers often dimerous to trimerous, inferior bilocular uniovulate ovary with gynobasic style, nut-like fruit with elaiosome, etc. (Rutishauser et al., 1998). Therefore, *Theligonum* either used to be considered as a small satellite monogeneric family called Theligonaceae (Cronquist, 1981; Takhtajan, 1997) or was included in the tribe Theligoneae within the Rubiaceae–Rubioideae (Thorne, 1992; Robbrecht, 1993a; etc.). Molecular data have shown this species to be at the base of the Rubieae (e.g. Bremer, 1996; Natali et al., 1996; Bremer and Manen, 2000; Fay et al., 2000; Anderson et al., 2001). The present study shows close similarities in the leaf fatty acid composition and lipid metabolism (i.e. presence of prokaryotic pathway of plastid lipids) of *Theligonum* and Rubieae.

Finally, Anthospermae show a more complex subtribal division based on their leaf fatty acid composition. Indeed, the genera *Anthospermum* and *Phyllis* are rich in $C_{16:3}$ fatty acid. Molecular data strongly suggest that both belong to the Anthosperminae subtribe (Anderson et al., 2001). Since the genera *Phyllis* is from Africa and the presence of the prokaryotic lipid biosynthetic pathway is considered to be an ancestral trait, our results do not contradict the suggestion of an African origin of the Anthospermae (Anderson et al., 2001). In contrast, *Coprosma* and *Nertera* (subtribe Coprosminae) are 18:3-plants. This is in agreement with *Rbc*L sequence data analyses (Bremer, 1996) that evidenced that the genera *Anthospermum* and *Phyllis* form a sister clade with Coprosminae and Opercullariinae. Molecular data suggest that Anthospermeae take the most basal position in a clade including Argostemmateae, Paederieae, Theligoneae and Rubieae; Paederieae are paraphyletic and form with Theligoneae a grade basal to the Rubieae (Bremer, 1996; Natali et al., 1996; Andersson and Rova, 1999; Bremer and Manen, 2000). Neither *Mycetia longifolia* (Argostemmateae), nor the three genera of Paederieae analyzed in this study (Table 1) are 16:3-plants, and the occurrence of 16:3-plants only concerns two branches, Anthospermeae–Anthosperminae and Theligoneae/Rubieae, which do not appear to be especially basal in cladograms, the latter being the most derived tribes within the Rubioideae (Bremer, 1996; Manen and Natali, 1996; Natali et al., 1996; Bremer and Manen, 2000; Anderson et al., 2001). Since the prokaryotic lipid biosynthetic pathway as an ancestral character would have been expected to occur either at relatively basal branches or at many branches of the cladograms, our results suggest two independent origins of 16:3-plants in Rubioideae. Additional investigations are obviously needed to investigate a putative common root in Rubiaceae.

## 4. Experimental

### 4.1. Material

Solvents used were of analytical grade. Sodium [1-$^{14}$C]acetate (53.9 Ci/mol) was obtained from CEA (Saclay, France). All other reagents were from Sigma Chemical Co. (St-Louis, MO, USA). Thin-layer chromatography plates were HPTLC silicagel 60 plates (Merck 60 F254).

### 4.2. Plant material

Samples of Rubiaceae fresh leaves were collected from plants grown in greenhouses (botanical garden of Nancy in August 1998, Arboretum of Chèvreloup in October 1998, National Botanic Garden of Belgium in June and September 1999 and botanical gardens of Iasi and Talence) or outside (botanical garden of Bordeaux in September 1999 and in the wild around Bordeaux). They were kept frozen before analysis.

### 4.3. Fatty acid analysis

Fatty acids of Rubiaceae leaf lipids were analyzed by GLC after conversion to the corresponding methyl esters. The procedure, described by Browse et al. (1986), used hot methanolic $H_2SO_4$ to digest fresh leaves and simultaneously convert the fatty acids to methyl esters. Samples were heated 1 h at 80 °C in 1 ml of methanolic $H_2SO_4$ (2.5% v/v). After cooling, 300–600 ml hexane and 1.5 ml $H_2O$ were added. Fatty acid methyl esters (FAMES) were extracted into the organic phase by vigorous shaking and a two-phase system was established by centrifugation (1500*g*, 10 min). An aliquot of the hexane phase (1 μl) was analyzed with a gas chromatograph (Hewlett-Packard 5890 series II) fitted with a flame ionization detector and electronic integrator (Hewlett-Packard 3396 series III). The column was programmed for an initial temperature of 160 °C for 1 min, followed by a 20 °C min$^{-1}$ ramp to 190 °C and a secondary ramp of 5 °C min$^{-1}$ to 210 °C. This final temperature was maintained for a further 5 min. The retention times of FAMES were determined by comparison with standards (Sigma Chemical Co., St. Louis, MO, USA). Several analyses of the same sample led to similar results.

### 4.4. Sodium [$^{14}$C]acetate incorporation in Rubiaceae leaf lipids and analysis of labeled lipids

Five μl of sodium [1-$^{14}$C]acetate (86 nCi) diluted in 10 μl of deionized water were applied for 30 min on

leaves of *Phuopsis stylosa*, *Coffea arabica* and *Pentas lanceolata*. Leaves were then rinsed with water and lipids were extracted according to the procedure described by Mongrand et al. (2001). Individual lipids were then purified by one-dimensional TLC using the solvent system described by Vitiello and Zanetta (1978). Lipids were located by spraying the plates with a solution of 0.001% (w/v) primuline in 80% acetone, followed by visualization under UV light. The radioactivity associated with the lipids was determined using a PhosphorImager cassette, and signals were quantified using ImageQuaNT software (Molecular Dynamic).

### 4.5. Statistical analyses

A non-normalized PCA (Jobson, 1992b) was carried out. The Box–Cox transformation $y = (x^a - 1)/a$ was performed on the 13 variables corresponding to fatty acids with $a = 1/2$ (Jobson, 1992a). Among the 107 Rubiaceae species, nine (no. 6, 13, 15, 30, 32, 34, 45, 71 and 94) were omitted during the calculation process because of their very peculiar profile. *Mycetia longifolia* (no. 1), which possesses raphides, is considered as an Isertieae by Robbrecht, but was transferred to Rubioideae for statistical analyses in agreement with cladistic data (Bremer, 1996). Ward hierarchical clustering (Ward, 1963; Jain and Dubes, 1988) was attempted on the species described by the first 10 principal components. The results were obtained with the Data Analysis software SPAD (Lebart et al., 1996).

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