Patterns of Fatty Acid Composition in Seed Oils of *Cuphea*, with New Records from Brazil and Mexico.

Graham, S.A.^{a*}, Pinheiro Coelho José, G.^b, Murad, A.M.^b, Rech, E.L.^b, Cavalcanti, T.B.^b

^aMissouri Botanical Garden, 4344 Shaw Blvd., Saint Louis, MO 63110, USA.

^bEMBRAPA-Cenargen, Final W5 Norte, CP 02372, 70770-900, Brasília, D.F. Brazil.

*Corresponding author. Tel. +01 314 577 9473 ext. 6206.

E-mail addresses: shirley.graham@mobot.org; gupcj@hotmail.com;

andre.murad@embrapa.br; elibio.rech@embrapa.br; taciana.cavalcanti@embrapa.br

Abstract

Herbaceous flowering plants of the genus *Cuphea* (Lythraceae) are noted for synthesizing significant amounts of medium-chain caprylic, capric, lauric, and myristic fatty acids (FAs) in the seed oils, with each species typically emphasizing one of several FAs. The genus is a prospective temperate source of oilseeds for fatty acids used in the manufacture of products as diverse as soaps, food, and biofuels, and it has become a model research system for the study of enzyme-mediated fatty acid synthesis. Seed oil FA composition for 44 species is reported here, including first records for 25 species from South America, primarily from Brazil. Nineteen species are re-analyzed to verify earlier results and expand information on variability in oil composition. An appendix summarizes seed oil composition of 108

species classified in two subgenera and in 10 of 13 sections. Fifty percent are lauric-acid (C12:0) producers. In South America, the most common FA dominating *Cuphea* oils is lauric acid. In North America, a secondary center of speciation, seed oils are more diverse; lauric and capric acid (C10:0) are most common, but caprylic (C8:0), myristic (C14:0), linoleic (C18:2), and linolenic acid (18:3) predominate in some species. The dominant FA in the seed oil of each species remains constant throughout its range and in differing habitats, and varies within a species in percentage of total FAs 14-17%. Factors affecting variation were briefly examined. The evolutionary pattern of the major seed oil FAs was explored using data from 61 species mapped to a molecular-based phylogenetic tree. Three significant divergent events indicate changes in seed oil composition were influential in the adaptive radiation and speciation of *Cuphea*. The basal dichotomy of the genus separates subg. *Cuphea* with linoleic acid (C18:2) as dominant FA from subg. *Bracteolatae* with myristic and lauric acids dominant. Evolution of the dominance of capric acid (C10:0) occurred following introduction of the genus into North America. Greatest diversity of seed oils evolved in North America in the most derived clade in the genus, where FAs from caprylic acid (C8:0) to linolenic acid dominate among the species.

Keywords: *Cuphea*, oilseeds, fatty acids, gas-chromatography mass spectrometry, adaptive radiation

1. Introduction

Cuphea is a monophyletic American genus of the Lythraceae with about 240–250 species of herbs and subshrubs, and an active focus of research into new sources of seed oils (Graham, 1989a; Knapp, 1993; Phippen et al., 2006; Cermak et al. 2015). The genus is considered unique in the family for the production of seed oils emphasizing medium-chain saturated fatty acids (FAs). Of the 28 genera in the family, 22 have known seed oil composition; all are dominated by linoleic acid (C18:2), as in the majority of angiosperms oilseeds (Browse, 1997), except *Cuphea* (Graham & Kleiman, 1987; Melgarejo et al. 1995). In most *Cuphea*, seeds, instead, synthesize high percentages of the short and medium carbon-chain fatty acids caprylic (C8:0), capric (C10:0), lauric (C12:0), or myristic acid (C14:0) (Graham and Kleiman, 1987). Each of these FAs and their triglycerides exhibit specific properties important in the manufacture of detergents, lubricants, food products, and specialty chemicals (Graham, 1989a; Graham et al., 1981; Graham and Kleiman, 1985, 1992). Depending on the species, one FA among the several present in the seed oil dominates the composition of the oil, and accounts for about 50-85% of the total FA constituents. The oils occur in the plant embryo, which is commonly 30-33% oil content by weight (Thompson & Kleiman, 1988).

Research on *Cuphea* oilseeds began in the 1970's by a consortium of agronomists and industrial chemists aiming to develop a temperate source of lauric acid as an alternative to sources from petroleum and imported tropical coconut and palm kernel oils, which pose significant supply and environmental problems (Hirsinger, 1980; Knapp, 1993). Palm oil production, in addition to being restricted to the tropics, requires large mono-cultural plantations, which are subject to a host of destructive diseases, making the supply of oils uncertain (Pomsuriya, et al. 2013; Hushianian et al. 2013). Further, the expansion of palm cultivation has led to accelerated deforestation around the world, and is an increasing threat

to the rich, irreplaceable biodiversity of tropics (de Franqueville, 2003; Fitzherbert et al. 2008; Turner et al. 2011).

There are challenges to developing *Cuphea* as a temperate crop, but major advancements have been made in recent years, especially in control of seed germination (Cernac et al., 2006; Volk et al., 2006; Berti et al., 2008), prevention of seed shattering (Knapp, 1993; Yanofsky, 1998; Knapp and Crane, 2000), and in improved crop management practices (Berti & Gesch, 2015). As new tools of technology have emerged, prospects have increased for commercial applications of *Cuphea* oils in the specialty oil market, in areas as diverse as biofuels and herbicides (Vaughn & Holser, 2007; Knothe et al., 2009; Lovestead et al., 2010; Kim et al., 2015), cosmetics (Florence et al., 2012; Cermak et al., 2015), pharmaceuticals (Bertetti et al., 2012), nutrition (Zentak et al., 2011), and for manufacture of useful oil byproducts (Tisserat et al., 2012). Increased knowledge of the thioesterase enzymes involved in fatty acid synthesis in *Cuphea* is also advancing the possibilities for designer seed oils with specific compositions for specialized uses (Dehesh et al., 1996a; Filichkin et al., 2006; Jing et al., 2011).

To date, FA composition of *Cuphea* seed oils has been determined for 83 species (Hirsinger, 1980; Graham et al., 1981; Graham, 1989a; Wolf et al., 1983; Graham & Kleiman, 1985, 1992). The majority of these species are endemic to North America, including Central America. Seed oils have been analyzed for 53 of the approximately 78 North American species. The seed oil composition in *Cuphea* endemic to South America/Caribbean, the primary center of species diversity (Barber et al. 2010), is less well known. Thirty of approximately 162 South American species have been analyzed. In addition, only a few species such as *C. lanceolata*, *C. viscosissima*, *C. avigera* var. *pulcherrima*, and *C.*

wrightii from North America and none from South America, have been intensively investigated for commercial development (Berti and Gesch, 2015; Kim et al., 2015).

This study reports FA composition of *Cuphea* seed oil for 44 species from Brazil, Bolivia, Mexico, and Dominican Republic, including first-time analyses for twenty-five species. One first-time analysis is for an endemic species of Bolivia, and 24 are for endemic species of Brazil, which nearly doubles the number of species from South America investigated for seed oil composition. Nineteen species of *Cuphea* with previously published accounts are resampled, several from multiple collections, to expand information on infra-specific variation in oil composition and for general comparison with earlier results. Factors influencing the diversity in *Cuphea* seed oil are briefly discussed. The dominant FAs in 61 species are mapped to a previously published molecular-based phylogeny of the same species to examine the evolution of dominant fatty acids in the genus. A summary of seed oil composition patterns from this study and previous publications for 108 species is presented as an Appendix.

2 Materials and methods

2.1 *Cuphea* seeds

Mature seeds were obtained primarily from wild populations collected in Brazil by T. Cavalcanti, S. Graham, and G. Pereira-Silva, with others, through Embrapa Genetic Resources and Biotechnology's program of *Cuphea* seed collection and conservation in Brazil (Table 1). Seventeen species based on field collections from 1978 to 1989 and previously published between 1981 and 1992 were re-analyzed in the current study using

different seed sources. Two species, *Cuphea melanium* (sect. *Brachyandra*) and *C. avigera* var. *pulcherrima* (sect. *Diploptychia*), were re-analyzed employing seeds reserved from the original collections made in October, 1984, and November, 1989, respectively. Seeds were stored in paper at room temperature (25°C) prior to study. Viability was not directly relevant to the study, and was not tested. Seeds selected for analysis possessed an enlarged lens shape indicative of mature, oil-filled embryos. Three to five seeds were sampled for FA analysis. Voucher plant specimens are deposited in the Embrapa Genetic Resources and Biotechnology Herbarium (CEN) and the Missouri Botanical Garden Herbarium (MO). Voucher data are summarized in Table 1. The taxonomic classification of Koehne (1903) is employed as the organizational tool for the seed oil data and discussion.

2.2 Extraction and derivatization

Seed oils were extracted and derivatized according to a modified version of the procedure described by Primomo et al. (2002). Each sample was added to a glass flask and dried overnight in an oven at 90 °C. Samples were then crushed with glass rods and soaked in 500 µL of 0.5 mol L⁻¹ KOH-methanol solution, followed by heating in an oven 1 hr at 80 °C. After cooling to room temperature, 2 mL of 1 mol L⁻¹ H₂SO₄-methanol was added to the flasks and vortexed 15 seconds. Samples were again heated 1hr at 80 °C and cooled to room temperature. Two mL of n-hexane was added, vortexed one minute, and incubated at room temperature for 10 minutes until the phases separated. Gas chromatography autosampler vials were filled with 1 mL of the hexane supernatant for analysis.

2.3 GC-MS analysis

Gas-chromatography mass spectrometry analysis was conducted on a Shimadzu GCMS-QP2010 provided with an autosampler. An OmegaWax 250 capillary column (30 m ×

0.25 mm $\times$ 0.25 μ m) (Supelco Products, Sigma-24136) was subjected to an initial temperature of 50°C, increased at a rate of 4°C/min to a final temperature of 240°C, and maintained at final temperature for 10 minutes. Two μ L of each sample was injected at 250 °C. A linear flow of 2 mL/min was used with a helium pressure of 117.6 kPa, composing a total flow of 25 mL/min. Linear velocity was adjusted to 51.3 cm/s, purging at 3 mL/min with a split ratio of 10. The temperature of mass spectrometry operations was set at 250 °C; scan speed was 833 Hz ranging from 100-500 mass/charge ratio over a total period of 58 minutes per run. Standards of palmitic, stearic, oleic, linoleic, and linolenic acid were also derivatized and were run in the GC-MS for calibration. The percentage of each FA relative to the total FA content was calculated through area-under-the-curve measurements. Low abundance compounds are largely fragmented during the ionization process causing some ion types not to appear in the spectra. We choose the FA methyl ester peaks (10 peaks) with the highest ion confidence scores to approximate the total FA content. Gas chromatography profiles of derivatized oil extracts for five species of *Cuphea* exemplify some of the results (Fig. 1). Mass spectra were searched against the NIST 08 library using AMDIS software (Meyer et al., 2010).

2.4 Statistical Analysis

The composition is reported for FAs from C8:0 to C20:1. Results from multiple collections of the same species are presented as average $\pm$ standard error of the mean.

3. Results

3.1. Seed oil composition as related to taxonomic section and geography.

Results from 44 new analyses are summarized in Table 2. The Appendix summarizes previously published records of seed oil composition (Graham et al., 1981; Graham, 1989a; Wolf et al., 1983; Graham & Kleiman, 1985, 1992) plus the new and reanalyzed records presented here. Of the 108 species for which the seed oil composition is known, 54 (50%) are lauric-acid producers. In South America, the most common FA dominating *Cuphea* oils is lauric acid (C12:0). In North America, seed oils are more diverse. Capric acid is most common (23 spp., 41%), lauric acid is second-most common (15 spp., 27%). Caprylic (6 spp.), myristic (5 spp.), linoleic (5 spp.), and linolenic acid (2 spp.) are less frequent major FAs, together occurring in 32% of North American species.

The taxonomic classification of Koehne (1903), the only inclusive one available, consists of two subgenera and thirteen sections. The current analyses include representatives of two subgenera and ten sections. Three sections are not represented: sect. *Amazoniana* (Lourteig) Lourteig (17 species), sect. *Heteranthus* Koehne (8 species, 3 known from previous publications; see Appendix), and sect. *Melicyathium* Koehne (1 species).

Subgenus *Cuphea* (comprising about 25 spp.), is characterized by seed oils in which the dominant FAs have an 18 carbon-chain length and one or two unsaturated bonds. The species are herbs or sub-shrubs, less than 1 m tall, closely associated with wetlands or margins of rivers or temporary pools, and are widely distributed on both the North and South American continents. Two sections are recognized in the subgenus. In sect. *Archocuphea*, *C. circaeoides* has oleic acid (C18:1) as the dominant oilseed component ($48.4 \pm 3.7\%$). *Cuphea circaeoides* also comprises 12% arachidic acid (C20:0). *Cuphea pascuorum* (Table 2) and *C. mimuloides* are rich in linoleic acid (C18:2) (Graham et al., 1981).

In sect. *Cuphea*, linoleic acid is the dominant FA, averaging 50% of total FAs, and occurring in six of the seven species previously reported, including *C. racemosa* and *C.*

lindmaniana, which are re-analyzed here (Graham et al., 1981; Wolf et al., 1983; Graham & Kleiman, 1985, 1992). Oil composition in the seventh species, *Cuphea denticulata*, is dominated by oleic acid with 53.2 % of total FA content (Graham & Kleiman, 1992).

The much larger subgenus *Bracteolatae* (about 220 spp.) consists of perennial or annual herbs to small shrubs up to about 3 m tall, distributed in diverse wet to dry habitats at elevations from sea level to 4000 m. The most common dominant FA is lauric acid, which generally accounts for as much as 40–85% of total fatty acids in the oils. This subgenus, however, is notable for the wider array of oil composition patterns than occur in subg. *Cuphea*, and comprises species with the major FA either C8:0, C10:0, C12:0, C14:0, C18:2, or C18:3. Major fatty acids with longer carbon-chain lengths and unsaturated are rare in subg. *Bracteolatae*. Four species with linoleic acid predominating are exceptions: *C. linarioides* and *C. linifolia* in sect. *Euandra*, *C. subuligera* in sect. *Diploptychia*, and *C. flavovirens* in sect. *Melvilla* (Graham, in press; Wolf et al. 1983, then in sect. *Heterodon*). Two species are exceptions with linolenic acid (C18:3) as the major FA constituent: *C. purpurascens* in sect. *Heterodon* (Graham & Kleiman, 1982) and *C. spectabilis* in sect. *Diploptychia* (Wolf et al., 1983).

In subg. *Bracteolatae*, species with seed oils rich in lauric acid are primarily concentrated in five sections: *Heteranthus*, *Brachyandra*, *Euandra*, *Pseudocircaea*, and *Trispermum*. These are South American and Caribbean in distribution. Seed oil composition in Sect. *Heteranthus* (Lourteig, 1988) is known from three species. *Cuphea setosa* has 62 % lauric acid, and *C. epilobiifolia* and *C. tetrapetala* have 55% and 51% myristic acid, respectively (see Appendix). In sections *Brachyandra*, *Euandra*, *Pseudocircaea* (Lourteig, 1986) and *Trispermum* (Lourteig, 1987), lauric acid dominates in the oilseed. Exceptions are

214 *linarioides* and *C. linifolia* (C18:2, sect. *Euandra*), *C. strigulosa* (C14:0, sect. *Euandra*) and
 215 *C. sessilifolia* (C14:0, sect. *Trispermum*).

216 Species of three sections, *Heterodon* (Graham, 1988), *Leptocalyx* (Graham, 1989b),
 217 and *Diploptychia* (Graham, 1998) are primarily endemics in North America. Earlier studies
 218 summarized in the appendix, focused on seed oil composition in these sections. In sect.
 219 *Heterodon*, seed oil composition was previously determined for 25 of 28 species (Graham,
 220 1988, 1989a). Fourteen emphasized capric acid and nine emphasized lauric acid. Linolenic
 221 acid predominates in *C. purpurascens*, but the results may not be representative of the species
 222 because the seed, collected in 1911, may have substantially degraded. Two collections of *C.*
 223 *ferrisiae* produced different patterns with either capric or myristic acid predominant in the
 224 seed oil (Table 2; Appendix).

225 Seed oil composition in section *Leptocalyx*, known from five of seven species and
 226 two varieties, includes the same compositional patterns as in sect. *Heterodon*, with C10:0,
 227 C12:0, or C14:0 as the dominant FA. *Cuphea palustris* (now in sect. *Leptocalyx*, Graham et
 228 al., 1981) and *C. aequipetala* (Graham et al., 1981) share a common pattern in which myristic
 229 acid (C14:0) is the primary FA and caprylic acid (C8:0) is the second most abundant
 230 component of the oil. The flowers of the two species are closely similar, suggesting the
 231 species share a common ancestor (Graham, 1989b). Two novel thioesterases in *C. palustris*
 232 are known to act simultaneously within the same cell to synthesize C14:0 and C8:0 (Dehesh
 233 et al., 1996b). The pattern is seen elsewhere in the genus in the morphologically distant *C.*
 234 *schumannii* of sect. *Melvilla*.

235 Species of sect. *Diploptychia*, which are mostly endemic to Mexico (Graham, 1998),
 236 are the exclusive source of caprylic acid-rich seed oil. Of the eight species and one variety
 237 analyzed in the section, six are caprylic acid-dominant: *C. avigera* var. *pulcherrima*, *C.*

cyanea, and *C. ianthina* (Table 2; Graham & Kleiman, 1992), and *C. hookeriana*, *C. painteri*, and *C. pinetorum* (Graham et al., 1981). *Cuphea avigera* var. *pulcherrima* seed oil has the highest percentage of caprylic acid of any species tested, at 75% (Table 2) and 94.4% (Graham & Kleiman, 1992). In the rest of the section, *C. avigera* var. *avigera* and *C. nitidula*, emphasize capric acid (Graham et al., 1981; Graham & Kleiman, 1992), and *C. spectabilis* has 30.8 % linolenic acid (Wolf et al., 1983).

Section *Melvilla* is nearly equally represented by South and North American species. Of 42 species currently recognized in the section (Graham, in press), 19 are endemic to South America and 23 are endemic to North America. The seed oil composition of the South American species is still poorly known. However, first-time analyses for Brazilian species *C. egleri*, *C. grandiflora*, and *C. teleandra*, and Bolivian *C. splendida* found all are major lauric acid producers, as is the previously reported *C. melvilla* (Graham et al., 1981; Graham & Kleiman, 1985).

In the North American species of sect. *Melvilla*, 11 species and one variety have been analyzed. The seeds exhibit more diverse seed oil composition patterns than in South America. Four species emphasize capric acid, three lauric acid, two myristic acid, and two linoleic acid (Graham et al., 1981; Wolf et al., 1983; Graham & Kleiman, 1985, 1992; Appendix). One of three populations of *C. micropetala* produces oil with approximately equal amounts of capric, lauric, and myristic acid (Graham et al., 1981), whereas the other two populations emphasize myristic acid (Graham & Kleiman, 1992). Two populations of *C. subuligera* differ in the dominant seed oil FA; one, a purple-flowered form, emphasizes linoleic acid, the other, with orange-colored flowers (published under synonym *C. caeciliae*; Graham et al., 1981) is dominant in capric acid.

3.2. Comparison of results from previous published results.

In the re-analysis of 19 species, the major FA remained the same as previously reported (Table 3). The percentage of the major FA in the total composition varied, with 14 species showing a decrease and five species showing an increase in the percentage of dominant FA from previously known values. Percentage decrease among all re-analyzed species ranged from 3–24%, mean 17%. *Cuphea linarioides* (34.0 ± 11.2 % linoleic acid) exhibited exceptionally wide variation in percentage of the major FA in new analyses of three populations and a 45% decrease from a single earlier analysis; it was not included in the overall calculation in range of decrease. Percentage increases from earlier records ranged from 1–17 %, mean 8%. In the two species with seeds re-analyzed from the original collections after 35 and 40 years, *Cuphea avigera* var. *pulcherrima* showed a decrease in caprylic acid from the original results from 94.4% to 75 % and *Cuphea melanium* showed a decrease in lauric acid from 85.9% to 76.6%.

The majority of species yielded lower percentages of major FAs in the current analyses than in the previous studies. The differences may be attributed to a number of factors that are not specifically investigated in this study, such as seed age, differences in genetic or environmental response, or changes in methodology and equipment. In *Cuphea melanium* and *C. avigera* var. *pulcherrima*, the lower yield may be attributed in part to degradation of the seed oil through time.

3.3 Intraspecific variation.

The current analyses provide additional data points on intraspecific variation that could prove useful in the development of the genus for commercial purposes. Of the 44 new records, 16 are based on more than one collection. Five widespread and/or morphologically highly variable species are represented by new records from recently collected wild seed:

Cuphea ingrata (3 populations, C12:0, range 52.6–70.9%, mean $64.8 \pm 4.2\%$), *C. linarioides* (3 populations, C18:2, range 15.3–54.3%, mean $34.0 \pm 11.2\%$), *C. thymoides* (3 populations, C12:0, range 65.1–66.0, mean $65.4 \pm 0.2\%$), *C. ericoides* (5 populations, C12:0, range 31.1–53.2%, mean $43.2 \pm 3.5\%$), and *C. micrantha* (5 populations, C12:0, range 28.1–59.1%, mean $43.0 \pm 4.9\%$). In previous studies of wild collected seed by Ghebretinsae et al. (2008), three species, all widely distributed lauric acid producers, varied as follows: *C. strigulosa* (7 populations, range 42.9–61.8%, mean $52.8 \pm 8.2\%$); *C. carthagenensis* (12 populations, range 50.5–65.8 %, mean $58.6 \pm 5.2\%$), and *C. wrightii* (9 populations, range 48.2–60.5 %, mean $53.9 \pm 4.7\%$). The mean range of variability in percentage of the major FA for wild seed of the seven species (excluding the unusual results in *C. linarioides*) was 17%.

In studies of infraspecific variation in fatty acid composition of nine species under cultivation, Phippen et al. (2006) evaluated *Cuphea lanceolata* (60 accessions, capric acid variation 77–91% of total FA contents), *C. toluicana* (40 accessions, lauric acid variation 48–64 %), and *C. calophylla* (31 accessions, lauric acid variation 58–70%). The mean range of variability among the three cultivated accessions was 14%. Thompson et al. (1990) found lauric acid varied by 16% among cultivated individual plant progenies of *C. wrightii*. All studies suggest that development of other *Cuphea* species as a speciality oilseed source will benefit by initial extensive sampling of wild populations for infra-specific variation in seed oil composition, as well as testing of multiple progeny under different environmental parameters, to ensure highest oil yields.

4. Some factors affecting the diversity of seed oil composition in *Cuphea*.

4.1 Environmental and genetic influence.

The diversity in composition and yield of FAs in seed oils is generally accepted as the result of complex interactions between the genetic makeup of the parent plants and the environment, under natural selective pressures. In *Cuphea*, where the dominant FA of the seed oil remains constant, even in species exhibiting the widest environmental and distributional ranges in the genus, i.e., in *C. carthagenensis* and *C. strigulosa*, the major FA in the seed oil remains constant, indicating strong genetic control over the biosynthesis of FAs.

The effects of the environment on variation in the major FA of *Cuphea* seed oils have been investigated in *C. carthagenensis*, *C. strigulosa*, and *C. wrightii*. Ghebretinsae et al. (2008) found that in wild seed collected under differing temperatures, elevations, and latitudes, all factors affected seed oil composition, but interaction of the three, more any one factor alone, most strongly influenced the expression of the dominate fatty acid. In studies of cultivated *Cuphea* in the midwestern United States, Gesch & Forcella (2007) and Gesch et al. (2009) determined that temperature and water supply had significant effects on both FA percentages and total oil yield. Kim et al. (2011), examining variation in total oil content in two capric acid producers, *C. lutea* and *C. viscosissima*, and lauric acid producer *C. wrightii*, found oil content increased with decreasing latitude, and both temperature and moisture were critical factors in the regulation of oil content. Overall, *Cuphea* growth response appears to be optimum under temperate to sub-tropical conditions and abundant water supply. Species adapted to dry, hot conditions, as occur in the Brazilian savannas, cerrado, and caatinga, have not been considered likely candidates for domestication. Species of those areas may be possible sources of genetic adaptations for development of *Cuphea* suitable to cultivation in tropical, dry environments.

In recent decades, research on how the varied composition and accumulation of oil in oilseeds is controlled has expanded significantly. Several species of *Cuphea* have been useful research models aimed at clarifying the biosynthetic pathways to specific FAs and storage triacylglycerols, and on understanding how the regulating mechanisms respond to changing external conditions (Bafar et al., 1990, Dehesh et al., 1996a, 1996b; Geller et al., 1999; Schütt et al., 2002; Slabaugh et al., 2013).

4.2 Evolutionary factors affecting seed oil composition: phylogenetic history and adaptive radiation.

Given that oil seeds in flowering plants provide the energy needed for germination and growth, natural selection acting on genetic variation in seed oil composition may be expected to lead toward the most efficient and energy-rich oil composition needed for establishment of the progeny (Linder, 2000). The more than 240 species of *Cuphea* occupy a wide range of habitats and elevations and also exhibit an unusual diversity in seed oil composition, suggesting that changes in the major FA of *Cuphea* seed oil could have been a significant factor in the diversification of the genus. Hypothetically, the genetic variation in fatty acids, demonstrated in both wild seed and cultivated progeny, is sufficient for natural selection to act in adapting seed oil composition to a variety of micro habitats, where especially different temperatures could affect germination rates and the supply of energy provided by a particular oil composition.

Molecular-based phylogenetic analyses of *Cuphea* (Graham et al. 2006; Barber et al. 2010), although not yet species-inclusive, provide some insight into major divergent events in the history of the genus and allow preliminary examination of changes in fatty acid patterns of the seed oils along branches of the phylogenetic tree.

We utilize here the Bayesian majority rule consensus tree of 70 species of *Cuphea* by Barber et al. (2010) as a framework for examining the phylogenetic patterns of distribution of major FAs (Fig. 2). The tree was constructed based on three genetic markers, the nuclear rDNA internal transcribed spacer (ITS) and chloroplast trnL-F and rpl16 sequences. The outgroup consisted of three other genera of the family, *Pleurophora*, *Pehria*, and *Woodfordia*. Of 70 species treated in the phylogeny, the major fatty acid in the seed oil is known for 61. These were mapped to the phylogeny and the predominate FAs present in the terminal clades were summarized by clade (Figure 2).

The phylogeny identified three significant divergent events in the evolution of seed oil composition. An initial dichotomy at the base of the genus (Fig. 2, I, Barber et al., 2010), corresponds to the two taxonomic subgenera recognized in morphological and molecular-based phylogenies (Graham et al., 2006; Barber et al., 2010). It also corresponds to a change in the major FA in the seed oil from linoleic acid, the ancestral type present in the outgroup and in subg. *Cuphea*, to lauric acid and myristic acid in subg. *Bracteolatae*. Species of subg. *Cuphea* emphasize the same longer-chained, polyunsaturated fatty acids that dominate seed oils of the outgroup and angiosperm seed oils generally (Browse, 1997). The species-rich subg. *Bracteolatae* emphasizes shorter-chained, saturated FAs. Seven major clades evolved in a long grade following the initial dichotomy at the base of the family. The basal members of subg. *Bracteolatae* and two successive phylogenetic grades are lauric acid producers, with the exception of one species each in Clades 2 and 4 in which myristic acid (C14:0) is the dominant FA. Myristic acid also is dominant in one species each in North American Clades 5, 7, and 9. In the evolution of seed oil composition, the appearance of myristic acid as the dominant FA in the oil could have been gained twice, on the branches to Clades 2 and to Clades 4–8, and lost once on the branch to Clade 6. Alternatively, as in Fig. 2, myristic acid

could have been gained once on the branch to subg. *Bracteolatae* and lost twice, on branches to Clades 3 and 6.

A second important change in major FA is marked by the appearance of capric acid as the dominant FA following the introduction of a South American/Caribbean ancestor into the southern latitudes of North America (Fig. 2, II, Clade 5). Capric acid is not expressed Clades 6 and 7, but occurs again in Clade 8.

A third significant change in FA composition appears within the North American monophyly (Fig. 2, Clades 5–8) at the base of Clade 8. The clade includes 10 species and two varieties, exhibiting six different dominant FAs in the seed oil. Species with seed oils having caprylic acid (C8:0) and linolenic acid (C18:3) as major fatty acids appear for the first time in the phylogeny, along with species producing primarily linoleic, myristic, lauric, and capric acids, which earlier evolved as major FAs in the seed oils. Half of the clade members are capric acid producers. The clade includes one caprylic acid producer. Four additional morphologically similar species and one variety in sect. *Diploptychia* also have caprylic acid as the dominant FA. They are not yet known phylogenetically.

The phylogeny suggests that changes in the major FA of the seed oil played a significant role in the diversification of this monophyletic genus. The three major divergent events at nodes I, II, III represent textbook examples of adaptive radiation (Figure 2; Schluter, 2000). The most basal divergent event at Node I is associated with a change in seed oil composition from the ancestral linoleic acid to lauric acid, dominates the seed oils of 25 South American endemic species of a total of 27 comprising Clades 2, 3, and 4 that lead to Node II. Following introduction of *Cuphea* into North America, most likely by an ancestor from the *Brachyandra*/*Euandra* pool of species with lauric-acid seed oils, capric acid

appeared, currently exemplified by nine of the ten North American endemic species comprising Clade 5. The divergence at Node III leading to Clade 8 is particularly notable for the ten highly distinctive endemic species that appear in this most highly derived clade of the genus. They appear in association with the greatest diversity of seed oil patterns within any clade in the phylogeny.

The changes in dominant FA and associated radiation and terminal speciation events are interpreted as adaptive in nature because of the direct relationship of the specific properties of each FA to the environment. Because each FA has a different melting point, as storage triglycerols in the seed catabolize, maximum energy is released at different temperatures depending on the proportions the various FAs, and especially on the most abundant FA component (Linder, 2000). The most effective release of energy for germination from the storage triglycerols is under selective pressure in the environment. A selective change in the most abundant fatty acid in the seed oil in relation to a change in environment, as might occur following long-distance seed dispersal or with in situ topographic or climate changes is projected to enhance survival of new seedlings in the new environment.

Such changes in seed oil composition as related to environmental and climatic change, have clearly been an influential factor in the development of *Cuphea* as the most species-rich member of the Lythraceae. The majority of the endemic species of *Cuphea* in Brazil, the primary center of speciation, are lauric acid producers and today are members of the savannas, dry woodlands (cerrado) and, to a lesser extent, the drier thorn scrub (caatinga) of the eastern highlands, vegetative systems that first began to appear about the Middle Miocene (Graham, A., 2010). In Mexico, the second center of speciation, the endemic species are more often capric acid producers and occupy habitats in the generally cooler pine-oak zone of the western and southern mountain chains and the Transvolcanic Belt. These habitats

became available as the result of extensive volcanism and mountain building, also beginning in the Miocene and continue to some extent to the present time.

4. Conclusions

The results confirm and strengthen observations from earlier published analyses that *Cuphea* seed oils produce a diversity of medium-chain FAs and differ in the major FA constituent depending on the species. Knowledge of oil composition of South American *Cuphea*, especially from Brazil, the primary center of speciation, is substantially expanded by 44 new analyses. Lauric acid is the predominant FA in seed oils of species endemic to South America/Caribbean. The occurrence of so many lauric acid species in Brazil offers more possibilities for exploration of genotypes suitable to dry habitats like those of the Brazilian savannas, cerrados, and caatinga. In North America, capric acid is the most common predominant seed oil FA. The greatest diversity in *Cuphea* seed oil composition and the only *Cuphea* species with high amounts of caprylic acid in the seed storage oil occur in North America.

Knowledge of seed oil composition has added a new highly informative character for interpreting the evolutionary history of the genus. Observed patterns of dominant FA distribution among the species are influenced at the local level by genetics and environment and on the larger scale by their phylogenetic history. The major basal divergent event of the genus, established on evidence from morphology and strengthened by molecular phylogenetic analyses (Koehne, 1903; Graham et al., 2006; Barber et al., 2010), is congruent with a major shift in seed oil composition from predominance of longer carbon-chain unsaturated fatty acids in subg. *Cuphea* toward medium to short carbon-chain saturated fatty acids in subg. *Bracteolatae*. The phylogenetic analyses, though still preliminary, suggest that

changes in the dominant fatty acid in the seed oils were influential factors in the adaptive radiation and speciation in *Cuphea* on both South and North American continents. Increase in the knowledge of the phylogenetic patterns of seed oil composition in *Cuphea* can guide further research on the changes in chemical pathways that produce diversity in seed oils, and at a practical level, aid selection of the most appropriate species groups as breeding material for the development of specialized oilseed cultivars.

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Figure 1. Gas chromatography profiles for derivatized oil extracts from *Cuphea* species.

Figure 2. The dominant FAs of 61 species mapped to a Bayesian majority rule consensus tree of *Cuphea* adapted from Barber et al. (2010). The tree is based on molecular sequences of 70 species (FAs unknown for nine species), three genetic markers, and an outgroup composed of the Lythraceae genera *Pleurophora*, *Pehria*, and *Woodfordia*. The dominant FA in seed oils of the species comprising the eight terminal clades are shown for each clade. Clades 1–4 are South American in distribution; clades 5–8 are North American in distribution. Three divergent events important in the phylogeny of FAs in *Cuphea* are indicated by numerals I, II, and III, which map the first appearance of C12:0 and C14:0, later appearance of C10:0 following introduction of *Cuphea* into North America, and the subsequent extensive diversification of FAs in Clade 8. Taxonomic sections of species present in each clade are indicated as: ARC = *Archocuphea*, BRA = *Brachyandra*, CUP = *Cuphea*, Dip = *Diploptychia*, EU = *Euandra*, HET = *Heterodon*, HETR = *Heteranthus*, LEP = *Leptocalyx*, MEL = *Melvilla*, PSEU = *Pseudosilene*, TRI = *Trispermum*.

Appendix. Summary table of fatty acid composition in *Cuphea* seed oils organized according to the classification of Koehne (1903). New records in red, those from the following previous publications in black (Graham et al., 1981, 1989; Wolf et al. 1983;

653 Graham & Kleiman, 1985, 1992; Ghebretinsae et al., 2007). The dominant FA in seed oil
654 of each species is highlighted in blue. Section/number = number of species currently
655 accepted in each section/ number of species with recorded seed oil composition in the
656 section. Collectors: AOS = A. Scariot, TBC = T. Cavalcanti et al., GPS = G. Pereira Silva, GR
657 = S. Graham. Collections are vouchered in the herbaria of CENARGEN-EMBRAPA and the
658 Missouri Botanical Garden. x = trace, less than 1% or none.

659

Figure 1

662

663 Figure 2

Table 1. Voucher data for all oil seeds analyzed for this report. Species are listed by taxonomic section. The “Seed Year” refers to the year seeds were produced, whether they were gathered from the original collection, or from the most recent progeny of the original collection. Habitat refers to the habitat cited in the collection data if provided, or from the known vegetation type of the collection site. Geographical coordinates and elevation are taken directly from the collection data when available. The abbreviation “na” = not available.

Cuphea Species	Collection	Seed Year	Country	Lat.	Long.	Elev. m	Habitat
Sect. <i>Archocuphea</i>							
<i>C. circaeoides</i>	TBC 2469	1999	Brazil	-13.2497	-41.6636	na	cerrado
<i>C. circaeoides</i>	TBC 2350	1999	Brazil	na	na	na	cerrado
<i>C. pascuorum</i>	TBC 2485	2001	Brazil	-12.2667	-38.9667	500	lake margin
Sect. <i>Cuphea</i>							
<i>C. lindmaniana</i>	TBC 3875	2014	Brazil	-27.9213	-50.6238	928	swale

<i>C. racemosa</i>	TBC 3828	2014	Brazil	-23.8316	-48.1702	624	roadside
Sect. <i>Brachyandra</i>							
<i>C. calophylla</i>	TBC 3865	2014	Brazil	-26.1322	-50.3108	786	roadside
<i>C. calophylla</i>	AOS 350	1989	Brazil	-21.2258	-43.7736	1180	pasture
<i>C. carthagenensis</i>	TBC 3864	2014	Brazil	-26.2627	-50.6219	762	disturbed field
<i>C. carthagenensis</i>	TBC 3894	2015	Brazil	-16.5958	-42.9272	701	roadside
<i>C. melanium</i>	TZ 31877	2000	DomRep	18.910 N	-70.750	1220	pineland
Sect. <i>Euandra</i>							
<i>C. acinos</i>	TBC 3904	2015	Brazil	-17.25	-43.0869	594	roadside
<i>C. adenophylla</i>	TBC 3908	2015	Brazil	-17.0797	-43.2763	1326	white sand
<i>C. brachypoda</i>	TBC 316	1989	Brazil	-13.5789	-41.8114	1120	cerrado
<i>C. diosmifolia</i>	TBC 199	1989	Brazil	-18.2166	-43.6833	1030	cerrado
<i>C. ferruginea</i>	TBC 2492	1999	Brazil	-15.8525	-48.9592	1100	savanna
<i>C. ferruginea</i>	TBC 2495	1999	Brazil	-15.8525	-48.9592	1100	savanna
<i>C. glareosa</i>	TBC 2477	1999	Brazil	-12.5289	-41.5589	1000	roadside

<i>C. glutinosa</i>	TBC 3871	2014	Brazil	-27.305	-50.4591	1056	roadside
<i>C. ingrata</i>	AOS 339	1989	Brazil	-21.1833	-44.3333	na	river margin
<i>C. ingrata</i>	AOS 343	1989	Brazil	-21.0575	-44.0797	1080	savanna
<i>C. ingrata</i>	AOS 367	1989	Brazil	-20.6667	-42.5208	1350	savanna
<i>C. ingrata</i>	AOS 398	1989	Brazil	-20.2875	-43.5081	1500	rocky montane savanna
<i>C. linarioides</i>	AOS 264	1989	Brazil	-16.7686	-47.6136	1315	cerrado
<i>C. linarioides</i>	AOS 301	1989	Brazil	-19.75	-45.95	1220	cerrado
<i>C. linarioides</i>	TBC 3878	2014	Brazil	-28.8736	-50.9738	847	disturbed field
<i>C. linifolia</i>	TBC 3888	2014	Brazil	-25.7708	-49.7	1009	white sands
<i>C. pohlii</i>	AOS 268	1989	Brazil	-16.7686	-47.6136	900	pasture
<i>C. rupestris</i>	TBC 2494	1999	Brazil	-15.8525	-48.9592	na	cerrado
<i>C. sclerophylla</i>	TBC 3938	2015	Brazil	-18.5977	-43.9016	1272	rocky montane savanna
<i>C. sincorana</i>	TBC 2460	1999	Brazil	-13.6261	-41.3269	na	rocky montane savanna
<i>C. spermacoce</i>	TBC 2490	1999	Brazil	-15.7797	-47.9297	1100	cerrado
<i>C. spermacoce</i>	TBC 3770	2013	Brazil	-14.8794	-48.2333	1150	cerrado

<i>C. thymoides</i>	TBC 3788	2014	Brazil	-20.1030	-43.7527	1050	roadside
<i>C. thymoides</i>	AOS 302	1989	Brazil	-19.8236	-45.9044	1220	mound field
<i>C. thymoides</i>	AOS 329	1989	Brazil	-21.3169	-44.8711	990	mound field
<i>C. tuberosa</i>	TBC 3858	2014	Brazil	-26.4944	-52.0105	1063	grassy roadside
<i>C. urbaniana</i>	TBC 3870	2014	Brazil	-27.2766	-50.4377	1059	wet field
<i>C. urbaniana</i>	TBC 3872	2014	Brazil	-27.5366	-50.3758	854	wet field
Sect. <i>Pseudocircaea</i>							
<i>C. gardneri</i>	TBC 389	1989	Brazil	-13.7975	-47.4583	830	forest margin
<i>C. lutescens</i>	TBC 3910	2015	Brazil	-17.0797	-43.2763	1326	cerrado
<i>C. lutescens</i>	TBC 3942	2015	Brazil	-18.5722	-43.5927	1197	cerrado
<i>C. sessiliflora</i>	TBC 426	1989	Brazil	na	na	na	cerrado
<i>C. sessiliflora</i>	TBC 3902	2015	Brazil	na	na	na	cerrado
Sect. <i>Trispermum</i>							
<i>C. bahiensis</i>	TBC 2480	1999	Brazil	-11.55	-41.1561	1012	roadside
<i>C. bahiensis</i>	TBC 357	1989	Brazil	-11.37	-41.15	970	white sand with rocks

<i>C. bahiensis</i>	TBC 360	1989	Brazil	-11.23	-41.16	1190	white sand, campo rupestre
<i>C. bahiensis</i>	TBC 366	1989	Brazil	-11.35	-41.06	870	sand,campo rupestre
<i>C. brachiata</i>	TBC 3903	2015	Brazil	-17.25	-43.0869	594	disturbed land
<i>C. brachiata</i>	LBB 796	1989	Brazil	-16.4497	-39.0647	10	roadside
<i>C. ericoides</i>	TBC 3893	2015	Brazil	-16.5958	-42.9277	701	cerrado
<i>C. ericoides</i>	TBC 200	1989	Brazil	-18.2494	-43.6003	na	rocky montane savanna
<i>C. ericoides</i>	TBC 218	1989	Brazil	-18.2494	-43.6003	na	cerrado
<i>C. ericoides</i>	TBC 331	1989	Brazil	-13/5789	-41.8114	1150	cerrado
<i>C. ericoides</i>	TBC 337	1989	Brazil	-13.0053	-41.3708	na	cerrado
<i>C. flava</i>	TBC 2410	1999	Brazil	-13.8666	-41.7166	30	white sand, restinga
<i>C. micrantha</i>	AOS 270	1989	Brazil	-17.2222	-46.8747	860	roadside
<i>C. micrantha</i>	AOS 272	1989	Brazil	-17.7425	-46.1725	790	river margin
<i>C. micrantha</i>	AOS 303	1989	Brazil	-19.8236	-45.9044	1220	mound field
<i>C. micrantha</i>	AOS 386	1989	Brazil	-20.3778	-43.4161	880	cerrado

<i>C. micrantha</i>	TBC 472	1989	Brazil	na	na	na	na
<i>C. pulchra</i>	TBC 291	1989	Brazil	-13.5789	-41.8114	1030	stream margin
<i>C. pulchra</i>	TBC 371	1989	Brazil	-11.1806	-40.5183	460	cerrado
Sect. <i>Heterodon</i>							
<i>C. angustifolia</i>	GR 677	2001	Mexico	18.9032	-100.258	60	stream margin
<i>C. lophostoma</i>	US 534700	1994	Mexico	18.95	-99.6333	1800	low wet roadside
<i>C. tolucana</i>	US 534810	na	Mexico	na	na	na	na
Sect. <i>Leptocalyx</i>							
<i>C. palustris</i>	AN 13027	1983	Mexico	17.059	-96.7216	1555	wet field
Sect. <i>Diploptychia</i>							
<i>C.avigera</i> v. <i>pulcher.</i>	GR 1052	1989	Mexico	18.9032	-100.258	1500	dry hillside
Sect. <i>Melvilla</i>							
<i>C. egleri</i>	GPS 16188	2012	Brazil	-9.5005	-65.0430	130	shady forest
<i>C. grandiflora</i>	GPS 7467	2003	Brazil	-16.3280	-48.2072	820	river margin
<i>C. splendida</i>	GR 1109	2002	Bolivia	-17.464	-63.650	470	mound field

<i>C. subuligera</i>	BR 41116	1990	Mexico	16.5255	-41.7738	2300	cloud forest
<i>C. teleandra</i>	TBC 3896	2015	Brazil	-16.5436	-42.9488	892	cerrado

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Table 2. New analyses of 44 *Cuphea* seed oils expressed as percentages of total fatty acid composition. Species are arranged according to the taxonomic sections of Koehne (1903). Twenty-five species reported here for the first time are indicated by the name in boldface; 19 new analyses for species previously analyzed are in normal font. The symbol ‘n’ refers to the number of plant collections analyzed. The dominant fatty acid of each species is in boldface; a dash indicates < 1% fatty acid. If more than one collection was analyzed, the standard deviation is given for new analyses and the mean for previous analyses.

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<i>CUPHEA</i> SPECIES	n	SOURCE	8:0	10:0	12:0	14:0	16:0	18:0	18:1	18:2	20:0	20:1
SUBG. <i>CUPHEA</i>												
Sect. <i>Archocuphea</i>												
<i>C. circaeoides</i>	2	Brazil	—	—	—	—	6.3 ± 0.1	1.6 ± 0.2	48.4 ± 3.7	9.4 ± 1.0	12.2 ± 0.0	6.0 ± 1.2
<i>C. pascuorum</i>	1	Brazil	—	—	—	—	22.0	1.8	16.0	52.8	2.5	1.4
Sect. <i>Cuphea</i>												
<i>C. lindmaniana</i>	1	Brazil	—	—	0.6	0.3	16.4	0.7	25.6	51.8	1.3	1.1
<i>C. racemosa</i>	1	Brazil	—	—	—	—	19.1	1.2	15.8	57.2	2.1	—
SUBG.												

BRACTEOLATAE**Sect. *Brachyandra***

<i>C. calophylla</i>	2	Brazil	—	13.4 ± 7.2	62.1 ± 3.9	9.5 ± 6.0	4.1 ± 1.6	0.7 ± 0.1	3.1 ± 0.9	5.2 ± 1.6	—	—
<i>C. carthagenensis</i>	2	Brazil	—	5.8 ± 1.3	60.6 ± 2.0	14.8 ± 0.4	5.8 ± 1.6	1.0 ± 0.5	3.9 ± 0.0	4.6 ± 0.3	—	—
<i>C. melanium</i>	1	D..Rep.	—	3.9	76.6	9.7	2.3	0.3	1.9	3.2	—	—

Sect. *Euandra*

<i>C. acinos</i>	1	Brazil	—	1.2	64.2	20.5	2.8	—	4.1	2.7	—	—
<i>C. adenophylla</i>	1	Brazil	0.7	4.7	72.5	14.7	2.1	0.1	2.5	1.5	—	—
<i>C. brachypoda</i>	1	Brazil	0.6	2.5	47.2	17.9	6.4	0.8	7.7	15.8	—	—
<i>C. diosmifolia</i>	1	Brazil	—	16.6	64.8	10.3	2.4	0.2	2.6	1.5	—	—
<i>C. ferruginea</i>	2	Brazil	—	1.4 ± 0.0	54.8 ± 0.6	21.6 ± 0.7	5.0 ± 0.0	0.5 ± 0.1	5.1 ± 0.7	9.0 ± 0.3	—	—
<i>C. glareosa</i>	1	Brazil	—	1.7	49.3	26.4	6.0	0.6	5.5	8.7	—	0.3
<i>C. glutinosa</i>	1	Brazil	—	24.2	49.5	5.4	2.6	0.4	2.9	9.1	—	—
<i>C. ingrata</i>	4	Brazil	—	8.1 ± 3.3	64.8 ± 4.2	9.2 ± 1.7	5.3 ± 1.6	0.0 ± 0.2	3.1 ± 0.5	4.0 ± 1.2	—	—
<i>C. linarioides</i>	3	Brazil	—	—	1.3 ± 0.5	11.2 ± 4.2	28.5 ± 4.2	3.1 ± 0.4	16.3 ± 2.1	34.0 ± 11.2	—	—
<i>C. linifolia</i>	1	Brazil	—	—	0.4	4.2	21.4	2.9	19.0	48.8	—	—

<i>C. pohlii</i>	1	Brazil	—	—	43.7	12.3	10.1	1.5	4.1	4.1	—	—
<i>C. rupestris</i>	1	Brazil	—	5.9	54.3	21.8	5.2	0.6	4.3	6.1	—	—
<i>C. sclerophylla</i>	1	Brazil	0.6	4.1	66.5	15.1	4.0	0.2	2.2	6.0	—	—
<i>C. sincorana</i>	1	Brazil	—	1.2	38.5	19.1	9.0	1.4	10.6	18.7	—	0.3
<i>C. spermacoce</i>	2	Brazil	—	1.5 ± 0.2	49.3 ± 4.5	27.5 ± 7.3	6.5 ± 1.4	0.7 ± 0.1	4.5 ± 2.2	6.9 ± 2.9	—	—
<i>C. thymoides</i>	3	Brazil	—	7.7 ± 1.0	65.4 ± 0.2	9.6 ± 0.4	4.1 ± 0.0	0.4 ± 0.1	2.1 ± 0.6	4.0 ± 1.1	—	—
<i>C. tuberosa</i>	1	Brazil	—	11.4	56.4	5.0	6.4	0.8	8.6	7.8	—	—
<i>C. urbaniana</i>	2	Brazil	—	—	47.5 ± 15.1	11.4 ± 2.4	6.4 ± 2.4	—	7.8 ± 2.6	10.4 ± 1.6	—	—
Sect. <i>Pseudocircaea</i>												
<i>C. gardneri</i>	1	Brazil	—	10.7	68.0	10.0	2.9	0.2	2.5	4.6	—	—
<i>C. lutescens</i>	2	Brazil	—	1.5 ± 0.3	73.2 ± 1.5	16.1 ± 1.7	2.6 ± 0.0	0.1 ± 0.0	2.8 ± 0.5	1.7 ± 0.1	—	—
<i>C. sessiliflora</i>	2	Brazil	—	1.7 ± 0.4	63.6 ± 2.4	23.1 ± 4.5	3.5 ± 0.0	0.3 ± 0.0	3.6 ± 1.0	2.3 ± 0.7	—	—
Sect. <i>Trispermum</i>												
<i>C. bahiensis</i>	4	Brazil	—	1.3 ± 0.0	47.0 ± 0.9	23.7 ± 0.4	6.8 ± 0.3	0.8 ± 0.0	9.8 ± 0.3	8.2 ± 0.3	—	0.2 ± 0.1
<i>C. brachiata</i>	2	Brazil	0.8 ± 0	2.5 ± 0.0	46.6 ± 0.2	20.9 ± 0.0	7.0 ± 0.1	1.0 ± 0.0	11.5 ± 0.7	9.2 ± 0.6	—	—

<i>C. ericoides</i>	5	Brazil	—	1.3 ± 0.1	43.2 ± 3.5	17.5 ± 1.6	6.4 ± 0.6	1.0 ± 0.1	14.2 ± 1.6	13.3 ± 0.9	—	0.1 ± 0.0
<i>C. flava</i>	1	Brazil	—	1.1	42.5	32.8	7.9	0.8	8.3	4.1	—	—
<i>C. micrantha</i>	5	Brazil, Dom.Rep.	—	3.0 ± 1.0	43.0 ± 4.9	18.9 ± 2.3	10.0 ± 1.9	0.9 ± 0.2	9.4 ± 1.6	11.4 ± 2.8	—	—
<i>C. pulchra</i>	2	Brazil	—	2.0 ± 0.0	55.8 ± 0.0	19.9 ± 0.7	5.4 ± 0.0	0.8 ± 0.1	8.5 ± 1.0	5.8 ± 0.5	—	—
Sect. <i>Heterodon</i>												
<i>C. angustifolia</i>	1	Mexico	—	67.3	3.8	1.7	5.2	1.3	9.7	6.0	—	—
<i>C. lophostoma</i>	1	Mexico	2.6	62.1	4.1	3.0	6.7	0.8	8.1	8.6	—	—
<i>C. toluicana</i>	1	Mexico	—	14.8	52.7	6.9	4.0	0.4	4.5	10.0	—	0.3
Sect. <i>Leptocalyx</i>												
<i>C. palustris</i>	1	Mexico	6.6	0.5	1.8	71.0	10.5	0.3	3.7	4.2	—	—
Sect. <i>Diploptychia</i>												
<i>C. avigera</i> var. <i>pulcherrima</i>	1	Mexico	75.0	4.8	—	—	3.7	—	4.0	5.3	—	—
Sect. <i>Melvilla</i>												
<i>C. egleri</i>	1	Brazil	—	0.5	57.3	34.2	2.5	0.1	2.1	1.4	—	—
<i>C. grandiflora</i>	1	Brazil	0.2	7.1	62.4	15.1	4.2	0.4	4.0	5.1	—	—

<i>C. splendida</i>	1	Bolivia	—	4.2	51.0	8.9	5.9	1.0	9.1	14.4	—	—
<i>C. subuligera</i>	1	Mexico	9.1	—	4.6	2.4	18.0	1.3	13.8	28.8	—	—
<i>C. teleandra</i>	1	Brazil	—	2.9	71.4	18.1	2.1	—	2.6	1.1	—	—

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680 Table 3. The percentage of the major FA present in seed oil of 19 species currently
 681 reanalyzed compared to previous analyses of the same species. When more than one
 682 plant collection was analyzed, superscripts indicate the number of collections used to
 683 calculate the standard deviation (current results) or the mean (previous results).

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<i>Cuphea</i>	Major	% of Major Fatty Acid		
species	FA			
		Present	Previous	Original Sources of Previous
		Analyses	Analyses	Analyses
Sect. <i>Cuphea</i>				
<i>C. lindmaniana</i>	C18:2	51.8	55.3	Wolf et al, 1983
<i>C. racemosa</i>	C18:2	57.2	58.8	Graham et al. 1981
Sect.				
<i>Brachyandra</i>				
<i>C. calophylla</i>	C12:0	62.1 ± 3.9 ²	80.2 ⁴	Wolf et al. 1983; Graham & Kleiman, 1985
<i>C. carthagenensis</i>	C12:0	60.6 ± 2.0 ²	65.6 ¹⁷	Graham et al. 1981; Graham & Kleiman, 1985; Ghebretinsae et al., 2007
<i>C. melanium</i>	C12:0	76.6	85.9	Graham & Kleiman, 1992
Sect. <i>Euandra</i>				
<i>C. diosmifolia</i>	C12:0	64.8	64.0	Graham & Kleiman, 1985

<i>C. glutinosa</i>	C12:0	49.5	64.8 ³	Graham et al. 1981; Graham & Kleiman, 1985
<i>C. ingrata</i>	C12:0	64.8 $\pm$ 4.2 ³	68.8	Graham et al. 1981
<i>C. linarioides</i>	C18:2	34.0 $\pm$ 11.2 ³	62.1	Graham & Kleiman, 1985
<i>C. linifolia</i>	C18:2	48.8	62.5	Graham & Kleiman, 1985
<i>C. sclerophylla</i>	C12:0	66.5	59.7	Graham & Kleiman, 1985
<i>C. thymoides</i>	C12:0	65.4 $\pm$ 0.2 ³	55.7 ²	Graham et al. 1981; Graham & Kleiman, 1985

Sect.***Pseudocircaea***

<i>C. lutescens</i>	C12:0	73.2 $\pm$ 1.5 ²	70.9 ²	Wolf et al., 1983; Graham & Kleiman, 1985
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Sect.***Trispermum***

<i>C. micrantha</i>	C12:0	43.0 $\pm$ 4.9 ⁵	50.1 ²	Graham et al. 1981; Graham & Kleiman, 1992
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Sect. *Heterodon*

<i>C. angustifolia</i>	C10:0	67.3	80.4	Graham, 1981
<i>C. lophostoma</i>	C10:0	62.1	81.1	Graham et al., 1981
<i>C. toluhana</i>	C12:0	52.7	63.8 ³	Graham et al. 1981

Sect. *Leptocalyx*

<i>C. palustris</i>	C14:0	71.0	63.7	Graham et al. 1981
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Sect.

Diploptychia

<i>C. avigera</i> v.	C8:0	75.0	94.4	Graham & Kleiman, 1992
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pulcherrima

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