Wilczekra, a New Genus of Celastraceae for a Disjunct Lineage of Euonymus

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Abstract—Disjunct distributions in poorly defined taxa can serve as indicators that the members of the isolated ranges are polyphyletic, as has been previously demonstrated in many plant families. Such taxa should be prioritized for inclusion in phylogenetic analyses so that at least one member from each isolated range should be sampled together with other closely related taxa. We describe a new monotypic genus (Wilczekra) for Euonymus congolensis, which is the only species of Euonymus reported in mainland Africa other than one other species (E. latifolius) whose range extends from Europe into northern Africa. We infer that Wilczekra congolensis is the sole African member of an early-derived lineage of Celastraceae that is most diverse in the Austral-Pacific but also includes Crossoptetalum from the West Indies and tropical America.

Keywords—Congo, Crossoptetalum, Euonymus congolensis, Gabon, phylogeny, polyphylectic.

Disjunct species distributions, as defined by Alphonse de Candolle, are those species that “…live in separate areas that are sufficiently isolated so that a current dispersal from one to the other area seems impossible” (Mayr 1982, p. 444). Disjunct taxa may be the product of the natural processes of dispersal or vicariance (Lyell 1832; Darwin 1859; Wallace 1869), but they may also be artifacts for taxa whose only similarities represent symplesiomorphy or convergence (Wen 1999). Taxa whose only similarities are plesiomorphic are expected to be paraphyletic while those whose similarities are convergent are expected to be polyphylectic (Hennig 1966).

As part of best phylogenetics practice, disjunct taxa in poorly defined lineages (or those that have at least one unique character state relative to species from other areas) should be prioritized for inclusion in phylogenetic analyses such that at least one member from each isolated range should be sampled together with other closely related taxa. By doing so, one may test whether the disjunct distribution is an artifact or the product of a biogeographic process. Disjunct distributions have been identified as artifacts of polyphyletic groups in a range of plant lineages including Sisymbrium L. (Brassicaceae; Warwick et al. 2002), Pinguicula L. (Lentibulariaceae; Cieslak et al. 2005), Cryptotaenia DC. (Apiaceae; Spalik and Downie 2007), Andracine L. and Leptopus Decne. (Phyllanthaceae; Vorontsova et al. 2007), Mukia Arn. (Cucurbitaceae; Schaefer et al. 2008), Echinodium Poit. ex Cass. (Bryopsida: Echinodiaceae; Stech et al. 2008), and Aecimenes Ruiz & Pav. (Bromeliaceae; Sass and Specht 2010).

Disjunct Genera within Celastraceae—Within Celastraceae there are 12 genera that, as traditionally defined, have widely disjunct distributions across continents and for which species from each of the isolated ranges have been sampled together with closely related taxa in a phylogenetic analysis. Seven of these genera (Celastrus L., Cuerova Triana ex Miers, Elachyptera A.C. Sm., Elaeodendron Jacq., Loeoserelia A.C. Sm., Pristimera Miers, and Microtrops Wall. ex Meisn.) have largely been supported as natural groups (Islam et al. 2006; Simmons et al. 2008, 2012a, 2012b; Coughenour et al. 2011). Alternatively, five widely distributed genera with disjunct distributions (Euonymus L., Gymnosporia (Wight. & Arn.) Hook. f., Maytenus Molina (not including Gymnosporia), Prionostemma Miers, and Salacia L.) have been shown, at least in part, to be paraphyletic or polyphyletic groups (Simmons et al. 2001a, 2001b, 2008; Coughenour et al. 2010, 2011; McKenna et al. 2011). In each of these cases species from one or more disjunct ranges were found to be more closely related to other genera than they are to the rest of the species in the same genus.

One genus in Celastraceae that has a widely disjunct distribution across continents for which the disjunct species have not yet been sampled in a phylogenetic analysis is Hippocratea L. Three species are currently recognized in Hippocratea (Hallé 1962, 1986; Simmons 2004). Smith (1940, p. 353), contra Loeener (1942), asserted that Hippocratea “…seems to be exclusively American” and recognized H. volubilis L. as the only species. Hallé (1962) agreed with Smith’s (1940) narrow delimitation of Hippocratea, but retained two species from tropical West Africa within the genus — H. myriantha Oliv. and H. vignei Hoyle. Of these three species only H. volubilis has been sampled in a phylogenetic analysis (Coughenour et al. 2011). Although delimitation of some genera within Hippocrateoideae is problematic (Coughenour et al. 2011), we believe that the three species of Hippocratea are a natural group based on their morphological synapomorphies of having cymes with intercalary ramifications and petals with abaxial pubescence (in H. vignei and H. volubilis the longer pubescence is confined towards the apices).

Euonymus—Euonymus L. is broadly distributed, primarily in the Northern Hemisphere, with the center of diversity in Asia, and a much smaller number of species in North and Central America, Europe, Africa, Madagascar, and Australia (Ma 2001). The African + Madagascan species have a curious disjunct distribution with a single species (E. latifolius (L.) Mill.) that reaches from Europe and Asia into northern Africa (Blakelock 1951; Ma 2001); one species (E. congolensis R. Wilczek) that is endemic to the Congo Republic, Democratic Republic of the Congo (Zaire), and Gabon (Wilczek 1959, 1960; Villiers 1973; Ma 2001); and two species that are endemic to Madagascar (E. elaeodendroides Loes. and E. pleurostyloides (Loes.) H. Perrier). Simmons et al. (2012b) determined that the Madagascan species (to be recognized as Astrocasine ined.) are only distantly related to Euonymus s.s. and identified fixed differences in both the fruit and seed between these two lineages. Euonymus congolensis is the focus of this study and is herein transferred to a new genus.

Euonymus congolensis was first described by Wilczek (1959) as a new species in preparation for his treatment of Celastraceae in the Flore du Congo Belge et du Ruanda-Urundi (Wilczek 1960). Wilczek (1959) described this species without
further comment about its range, disjunct distribution relative to Euonymus, or unusual character states. Wilczek (1960) asserted that the species is the only representative of Euonymus in Africa (but see above for E. latifolius in northern Africa) and is “aberrant” relative to other species of Euonymus with respect to its cupular floral disc, which resembles that of Celastrus rather than other species of Euonymus.

To our knowledge, the only other publications on the systematics of E. congolensis were by Villiers (1973) in his treatment of Celastraceae for the Flore du Gabon, Ma (2001) in his revision of Euonymus (the latter was also summarized by Ma (2002)), and Savinov (2010) in a poster abstract. Villiers (1973) simply noted that the species also occurs in Gabon. Ma (2001) only examined a single specimen and assigned the species to Euonymus section Euonymus. Euonymus congolensis had previously been documented in the Democratic Republic of the Congo and Gabon. Based on this known distribution Ma (2001) predicted that the species also occurs in the Republic of the Congo. In contrast to Villiers (1973) and Ma (2001), Savinov (2010) asserted that Euonymus congolensis is not related to other species in the genus based on its cyathiform flowers, pear-shaped capsules, and aril form.

Based initially on the disjunct distribution and later upon the unique disc shape of Euonymus congolensis relative to the rest of Euonymus we hypothesized that this species may represent a separate lineage. We tested this hypothesis using a phylogenetic analysis that incorporated both morphological and molecular data. We generated sequences of E. congolensis for two nuclear gene regions (26S rDNA and the internal transcribed spacers (ITS; including ITS1, 5.8S, and ITS2) of rDNA), and two plastid loci (maturase K (matK) and trnL-F). These data were analyzed together with morphological characters as well as 18S rDNA, atpB, phyB, and rbcL sequences generated in previous analyses.

**Materials and Methods**

**Taxon Sampling**—Preliminary MAFFT ver. 6.5 (Katoh and Toh 2008a) alignments and parsimony gene-tree searches using 26S rDNA and matK (the two most conservative gene regions sampled) independently of each other were performed using the combined taxon sampling of Simmons et al. (2008, 2012b) and McKenna et al. (2011). In both cases the gene trees indicated that the closest relatives of Euonymus congolensis include Crossopetalum P. Browne, Periptergyia (Bail.) Loes., and Siphonodon Griff. The most thorough sampling of species from Crossopetalum, Siphonodon, and their close relatives (including the Austral-Pacific clade of Apaephyllum McGill., Brassania A.C. Sm., Denhamia, Dicarpellum, Dinghout R.H. Archer, Hedrаниthera F. Muell., Hexaspora C.T. White, Hypsophila F. Muell., Macgregoria F. Muell., Menepetalum, Psammonyma Diels & Loes., Stackhousia Sm., and Tripterococcus Endl.) was performed by Simmons et al. (2012b). Therefore, the taxon sampling used by Simmons et al. (2012b) served as the baseline with which to add Euonymus congolensis.

In an attempt to decrease phylogenetic ambiguity, 13 terminals from Simmons et al.’s (2012b) analysis that had >70% missing / inapplicable data amongst the parsimony informative characters in the simultaneous analysis (Klug 1989; Nixon and Carpenter 1996) were deleted. In all but three cases (Platyptercarpus tannyzygus Dunkley & Brench, Pottingeria acuminata Prain, and Torsilhasia variabilis (C. Wright & A. Gray) Krug & Urb.), at least one other representative of each of the genera remained in this study.

**Morphological Characters**—Euonymus congolensis was added to the morphological matrix from Simmons et al. (2012b) after deleting the 13 terminals cited above. This morphological matrix was originally derived from Simmons and Hedin (1999) and contains 54 parsimony informative characters (representing variation in vegetative and floral morphology; leaf, seed, and wood anatomy; pollen morphology; and chromosome number). Euonymus congolensis was scored by examining specimens at the Missouri Botanical Garden (MO, J, de Bruijn 827, G. McPherson 16037, 16179) in July 2011 together with the species descriptions by Wilczek (1959, 1960) and Villiers (1973).

**Molecular Methods**—Total genomic DNA was extracted from two MO herbarium specimens collected in Gabon. DNA from G. McPherson 16179 was extracted using the protocol described by Alexander et al. (2007). DNA from L. White 1082 was extracted using a combination of protocols from Couch and Fritz (1990) and Sto¨ver and Mu¨ller (2010). All forms were amplified with the following PCR protocol: an initial denaturation of 96°C preceding 10 cycles denaturation (96°C for 45 s), annealing (50–53°C for 30 s), and extension (72°C for 2 min), followed by 25 cycles of denaturation (96°C for 20 s), annealing (50–53°C for 30 s), and extension (72°C for 2 min).

Amplifications of the matK locus were split into two reactions, one using the primer combination trnK-710 (Johnson and Soltis 1995) and matK-R1 (Yokoyama et al. 2000) for the 5’ end, and the second reaction using matK-F1 (Yokoyama et al. 2000) and matK-8R (Steel and Vilgalys 1994) for the 3’ end. When the second primer combination did not amplify, primer matK-F3 (Yokoyama et al. 2000) was used in place of matK-F1. Intron trl-F and the intergenic spacer were amplified in two reactions using the combinations ‘c’ and ‘d’ for the 5’ end and ‘e’ and ‘f’ for the 3’ end (Taberlet et al. 1991). The ITS region was amplified with primers ITSA and ITSB (Blattner 1999). Amplifications of 26S rDNA were performed using the primers 26S1 and 950rev (Kozuh et al. 1998). Amplified products were purified using Qiagen PCR Purification Kits (Qiagen Inc., Valencia, CA). Purified PCR products were sequenced by the University of Chicago Cancer Research Center DNA Sequencing Facility using automated fluorescent sequencing with ABI DNA Analyzers (Life Technologies Corporation, Carlsbad, CA). The same primers used for amplification were also used for sequencing. All new sequences generated in this study have been deposited in GenBank as follows: matK rDNA JQ247019, ITS rDNA JQ247022, matK JQ247025, trnL intron JQ247021, trnL spacer JQ247023, L. White 1082: 26S rDNA JQ247018, matK JQ247024, trnL intron JQ247020. In the regions of overlap the matK sequences differed by a single nucleotide and the 26S rDNA and trnL intron sequences were each identical.

**Data Analysis**—Euonymus congolensis sequences were manually added to the data matrices of Simmons et al. (2012b) in MacClade ver. 4.03 (Maddison and Maddison 2001) using the similarity criterion for alignment (Zurawski and Clegg 1987; Simmons 2004c). A 15-bp apomorph matK coding-region inversion was inferred (and the reverse complement was scored) for E. congolensis between positions 199 and 219, as were two insertions (5-bp at positions 126–130, 2-bp at positions 753–754) in the trnL-F spacer, and a 1-bp insertion at position 130 in ITS. Due to the high divergence in some ITS regions, we used the MAFFT’s QJINS-Ins option, which considers inferred secondary structure of rDNA (Katoh and Toh 2008a). This sequence was used as a first pass to guide the E. congolensis ITS alignment.

No mutually well-supported (≥70% BS and JK) cases of incongruence were identified in any of the parsimony vs. likelihood (Felsenstein 1973) analyses of individual gene regions, plastid, rDNA, or simultaneous analyses by Simmons et al. (2012b). Therefore, for simplicity, our phylogenetic analyses were restricted to parsimony. Parsimony analyses followed the same methodology used by Simmons et al. (2012b). Briefly, gap characters were scored using modified complex indel coding (Simmons and Ochoterena 2000; Muller 2006) and alternative process partitions (Bull et al. 1993; 26S rDNA, ITS, combined rDNA, matK, trnL-F, combined plastid, and morphology) were analyzed to check for mutually well-supported incongruence between gene trees or coalescent genes (Doyle 1992, 1995). Equally weighted parsimony tree searches were conducted using 2,000 random addition tree-bisection (TBR) searches with a maximum of ten trees held for each search in each matrix in PAUP* 4.0b10 (Swofford 2001) and jackknife analyses (JK; Farris et al. 1996) were conducted with the probability of appearing in a TBR cycle, 100 JK replicates were performed using TBR, and the resampling emulated. One thousand JK replicates were performed with 100 random addition TBR searches (each with a maximum of ten trees held) per replicate. All parsimony analyses were run while collapsing branches with a minimum possible optimized length of zero to increase tree-search efficiency (Davis et al. 2005) and decrease the potential for inflated jackknife values (Stotoros et al. 1991; Trees were printed using TreeGraph 2 (Stöver und Müller 2010). Support values were mapped onto the parsimony strict consensus tree so as to help minimize frequency-within-replicates (Davis et al. 1998) and undersampling-within-replicates.
Results

A simplified version (wherein selected genera that are not the focus of this study are represented by single terminals) of the simultaneous-analysis parsimony strict consensus tree of all eight gene regions and morphological characters is presented in Fig. 1 with parsimony JK values ≥ 50% above each branch. The complete tree is presented in Fig. S1 as supplemental online data. Equivalent trees for each of the seven partitioned analyses are presented in Figs. S2-S8.

In the 25S rDNA gene tree (Fig. S2), both accessions of Euryonymus congolensis were resolved as sister to Crossopetalum with 59% JK. In contrast, the single accession of E. congolensis that was sampled for ITS was resolved as sister to the clade of Orthosphenia + Rzedoessia in the ITS gene tree (Fig. S3), albeit with < 50% JK for that clade and the other three clades that separate it from Crossopetalum. In the combined rDNA tree (Fig. S4), both accessions of E. congolensis were resolved as part of a 12-way basal polytomy that also includes Crossopetalum. In the matK gene tree (Fig. S5), both accession of E. congolensis were resolved as sister to Peripterygia marginata (Baill.) Loes. with 61% JK; this clade is a member of a 58-way polytomy that includes Crossopetalum. In the trnL-F gene tree (Fig. S6), both accessions of E. congolensis were resolved as members of a five-way polytomy together with Crossopetalum, Peripterygia, Siphonodon, and the Austral-Pacific clade. In the combined plastid tree (Fig. S7) the one accession of E. congolensis sampled in this analysis was resolved as sister to Peripterygia marginata with 63% JK. In the morphology-based tree (Fig. S8), E. congolensis was resolved together with other species of Euryonymus, but this and all other clades along the “backbone” of the tree received < 50% JK.

In the simultaneous-analysis tree, both accessions of E. congolensis were resolved as sister to Crossopetalum with 70% JK (Fig. 1). This clade is a member of a four-way polytomy with Peripterygia, Siphonodon, and the Austral-Pacific clade. The clade that includes this polytomy received 78% JK. The Euryonymus congolensis ITS sequence is highly divergent from all other members of Celastraceae in the ITS1 and ITS2 regions (but not the 3’ end of 185 or the 5.85 region). The sequence was difficult to align to other members of Celastraceae, but the top BLAST (Altschul et al. 1997) hits are Celastraceae and it does not have an unusual GC content. After exclusion of the E. congolensis ITS sequence, the two clades cited above received 86% and 89% JK, respectively (Fig. S9). Euryonymus s.s. together with Glyptopteridale were resolved as an unambiguously supported clade (100% JK) that is highly divergent from the clade of E. congolensis + Crossopetalum, being separated from it by five other branches with 56%, 98%, 98%, 100%, and 78% JK in the simultaneous analysis (Fig. 1).

Discussion

Given that Euryonymus congolensis is only distantly related to Euryonymus s.s. (Fig. 1), and is distinct from its closely related genera (Crossopetalum, Peripterygia, and Siphonodon) phylogenetically, geographically, and morphologically, we erect a new monotypic genus (Wilczekra) for this species. The resolution of Wilczekra congolensis as the sole African member of a primarily Austral-Pacific lineage is unexpected both biogeographically and morphologically. But within this lineage the sister-group relationship of Crossopetalum (the sole member of the primarily Austral-Pacific lineage from the West Indies and tropical America) and Wilczekra is consistent with other disjunct plant lineages in the West Indies and central-West Africa (Duchen and Renner 2010; Michalak et al. 2010). Yet Crossopetalum is morphologically divergent from Wilczekra by having a 4-merous (rather than 5-merous) perianth, one ovule (rather than two) per locule, drupes (rather than capsules), and exarillate (rather than arillate) seeds.

We were only able to identify a single morphological or indel synapomorphy for the clade of Crossopetalum + Wilczekra — having opposite or whorled rather than alternate leaves. Yet alternate leaves are also present in some species of Crossopetalum (e.g. Edwin and Ding Hou 1975) and opposite leaves have been independently derived at least nine times within the Celastraceae, including Euryonymus s.s. The only morphological or indel synapomorphy that we identified for the clade of Crossopetalum + Peripterygia + Siphonodon + Wilczekra + the Austral-Pacific clade was a 6-bp insertion at positions 102–107 of the trnL-F spacer.

The recent segregation of Astrocassine, Dinghoua, and now Wilczekra from Euryonymus results in a well-defined Euryonymus lineage that is geographically and morphologically cohesive. Euryonymus no longer has disjunct distributions in Madagascar or Central Africa and is now restricted to North and Central America, Europe, northern Africa (for E. latifolius), Asia, Malesia, and Queensland in Australia (for E. australianus F. Muell.; Jessup 1984; Ma 2001). Euryonymus is now more homogeneous morphologically as well. Euryonymus s.s. has cymose or solitary flowers (vs. appearing as though racemose or thyrsoid in Dinghoua) annular discs (vs. cupular in Wilczekra), generally two ovules per carpel (vs. eight in Dinghoua), capsular fruits (vs. indehiscent in Astrocassine), and glabrous arils that at least partially surround the seed (vs. arils absent in Astrocassine, arils puberulent and developed along one side of the seed in Dinghoua; Ding Hou 1975; Ma 2001; Simmons et al. 2012b).

Taxonomic Treatment

Wilczekra M.P. Simmons, gen. nov.—TYPE SPECIES: Wilczekra congolensis (R.Wilczek) M.P.Simmons (= Euryonymus congolensis R.Wilczek).

The new genus differs from Euryonymus L. by its cupuliform rather than planar flowers and a cupular rather than annular disc; it also differs from most species of Euryonymus by its stamens that are inserted on the disc margin rather than on top of the disc and longitudinally rather than obliquely dehiscent anthers; it differs from Crossopetalum P. Browne, to which it is closely related, by having a 5-merous rather than 4-merous perianth, 2(3) ovules per carpel rather than 1, capsular fruits rather than drupes, and arillate seeds rather than exarillate seeds.


Fig. 1. Simplified simultaneous-analysis parsimony strict consensus tree with jackknife values ≥ 50% above each branch. The six clades separating *Euonymus congolensis* from *Euonymus* s.s. are indicated by filled triangles.
Eymology—Wilczekra is named after Rudolf Wilczek (1903–1984), a Polish botanist who worked at the National Botanic Garden of Belgium and completed several family-level treatments for the Flore du Congo Belge et du Ruanda-Urundi, including Celastraceae in 1960. In addition to describing many species of Celastraceae (including Euonymus congoensis), Wilczek (1956) first described the genera Apodostigma R.Wilczek and Bequaertia R.Wilczek. The monotypic fungal genus Wilczekia was proposed by Charles Meylan in 1925, but the species was transferred to Didelma by Kowalski (1975). Therefore, rather than naming the new genus “Wilczekia” using the standard convention (McNeill et al. 2007, recommendation 60B.1), an alternative spelling was adopted that includes Rudolf Wilczek’s first initial.

Ecology—Within the Kasai Sector of Province Kinshasa within the Democratic Republic of the Congo, Wilczekra congoensis is restricted to the central region where it is a dominant species that grows as small trees in semi-evergreen wet forests (Ayingwe 2001).

Comments—Savinov (2010) asserted that Wilczekra congoensis is not related to other species in Euonymus based on its cyathiform (a.k.a., cupuliform) flowers, pear-shaped capsules, and aril form. Regarding cupuliform flowers, both Wilczek (1959, 1960) and Villiers (1973) explicitly described the floral discs as cupuliform, but not the entire flowers. Having said that, the figures of W. congoensis in both Wilczek (1960) and Villiers (1973) do show cupuliform flowers. Likewise, although this may be an artifact of pressing, the flowers appear cupuliform in G. McPherson 16179 and L. White 1082 at MO. Based on this evidence we infer that Savinov (2010) is correct about W. congoensis having cupuliform flowers and, to our knowledge, this does indeed distinguish the species from all species of Euonymus s.s.

Regarding Savinov’s (2010) assertion that W. congoensis has pear-shaped capsules, Villiers (1973) described them as ovoid whereas Wilczek (1959, 1960) did not describe the capsule shape. In any case, this trait does not appear to distinguish W. congoensis from all species of Euonymus as ovoid capsules are present in many species of Euonymus (Ma 2001). Finally, Savinov (2010, p. 451) noted that seeds of W. congoensis “have a large, boat-shaped, yellow aril.” Species of Euonymus also have arils, some of which partially envelop the seed in a manner similar to W. congoensis. The aril shape in most species of Euonymus has been insufficiently described (e.g. Blakelock 1951; Ma 2001) to enable us to compare them with W. congoensis. Almost all species of Euonymus for which aril color has been reported have orange or red arils, though E. revoluta Wight has also been reported as having yellow arils (Ma 2001).

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