PHYLOGENY AND BIOGEOGRAPHY OF *TRIOSTEUM* (CAPRIFOLIACEAE)

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Abstract. *Triosteum* (Caprifoliaceae) includes three species from eastern North America and three from eastern Asia. The North American species and *Triosteum sinuatum* from Japan and northeastern China share axillary inflorescences and long calyx lobes. In contrast, the other two Asian species are characterized by terminal inflorescences and short calyx lobes. A sister group relationship between the Japanese and North American species would be congruent with a biogeographic pattern seen in several other groups (e.g., *Hamamelis*, *Viburnum* sect. *Pseudotinus*). We inferred the phylogeny of *Triosteum* on the basis of sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) and partial sequences of the gene for granule-bound starch synthase (GBSSI or *waxy*), using representatives of the other lineages of Caprifolieae for rooting purposes. In the combined data analysis, the North American species of *Triosteum* form a clade with the Chinese species, to the exclusion of the Japanese species, *T. sinuatum*. Axillary inflorescences and large calyx lobes may be ancestral in *Triosteum*, and changes to terminal inflorescences and small calyx lobes may have occurred along a branch showing very little molecular character change.

Keywords: Asia, biogeography, Caprifoliaceae, Caprifolieae, Internal Transcribed Spacer (ITS), North America, phylogeny, *Triosteum*, *waxy*.

Triosteum L. (Caprifoliaceae), a group of six species of perennial herbs, provides a fine example of the commonly observed intercontinental disjunction between eastern Asia and eastern North America (see Boufford and Spongberg, 1983; Wen, 1999). Three species of *Triosteum* occur in the eastern United States and Canada (Lane, 1954; Hara, 1983; Gleason and Cronquist, 1991): *T. perfoliatum* L. occurs from Massachusetts to southern Ontario and Minnesota, south to South Carolina and Oklahoma; *T. aurantiacum* Bickn. is found from Quebec and New Brunswick to Minnesota, south to Georgia, Kentucky, and Oklahoma; and *T. angustifolium* L. is distributed from Connecticut to southern Ontario, Ohio, and Missouri, south to North Carolina, Alabama, and Louisiana. We recognize three Asian species, following Hara (1983) (but cf. Lane, 1954): *T. himalayanum* Wallich (including *T. fargesii* Franch. and *T. erythrocarpum* H. Smith) occurs in the eastern Himalayas, Tibet, and western and central China; *T. pinnatifidum* Maxim. (including *T. intermedium* Diels & Graebn., *T. rosthornii* Diels & Graebn., and *T. maruyamae* Ohwi) is found in western and northern China and in central Honshu, Japan; and the much rarer *T. sinuatum* Maxim. is predominantly found in central Honshu, Japan, but is also known from several collections in northeastern China (Hara, 1983).

The Japanese species, *T. sinuatum*, is most similar to the North American species (Lane 1954; Hara 1983). Whereas the Chinese species have terminal, spike-like inflorescences and tiny calyx lobes (much shorter than the corolla) when in flower and persisting on the mature fruit, *T. sinuatum* and the North American species have three-flowered, axillary inflorescences and relatively long calyx lobes (as long as or longer than the corolla). A direct phylogenetic relationship between the Japanese species and the North American species would be consistent with findings in *Hamamelis* L. (Wen and Shi, 1999; Li et al., 2000) and in *Viburnum* L. sect. *Pseudotinus* (J. Li and M. Donoghue, unpubl. data).

We thank J. Li for verifying DNA sequences for *Lonicera* accessions, and other members of the Donoghue lab for advice and assistance. We are also grateful to David Boufford and Tatemi Shimizu for help in obtaining samples of *Triosteum sinuatum* from Japan, and to members of several expeditions to Sichuan, Qinghai, and Yunnan, China (DEB-9705795), for their help in the field, especially David Boufford, Ying Tsun-sheng, Yin Kai-pu, Wu Sugong, and Sun Hang. Phil Cantino kindly provided advice on the project and plant material for the North American species, which are being cultivated at Ohio University. This study was partially supported by a Putnam Postdoctoral Fellowship to KRG, provided through the Arnold Arboretum of Harvard University.

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Harvard Papers in Botany, Vol. 5, No. 1, 2000, pp. 157–166.

Here we present a phylogenetic analysis of *Triosteum*, designed specifically to test the hypothesis that *T. sinuatum* is related to the eastern North American species. In part, our analysis is based on sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA), as these often show phylogenetically informative variation in studies of interspecific relationships (Baldwin et al., 1995). ITS has already proved useful in studies of several other genera of Dipsacales, including *Weigela* Thunb. and *Diervilla* Miller (Kim and Kim, 1999), *Sambucus* L. and *Adoxa* L. (Eriksson and Donoghue, 1997), and *Viburnum* L. (Donoghue and Baldwin, 1993; Baldwin et al., 1995).

We have also investigated the phylogenetic utility of a portion of the gene for granule-bound starch synthase (GBSSI or *waxy*). Mason-Gamer et al. (1998) found single copies of *waxy* in a broad sampling of grasses (Poaceae). Single copies of this gene have also been reported in agriculturally important plant groups, including potato, pea, cassava, maize, barley, wheat, and rice (Shure et al., 1983; Klosgen et al., 1986; Rohde et al., 1988; Clark et al., 1991; van der Leij et al., 1991; Denyer and Smith, 1992; Dry et al., 1992; Salehuzzaman et al., 1993; Wang et al., 1995). Two copies of *waxy* have been found in the Rosaceae, however (Evans et al., 1998). With the entire gene containing 13 introns, *waxy* can provide potentially many more characters than the ITS region. A relatively high rate of sequence change in *waxy* introns has been documented, even where there is little ITS variation (e.g., Mason-Gamer et al., 1998).

Triosteum belongs to Caprifolieae (Caprifoliaceae sensu Backlund and Pyck, 1998; APG, 1998), which also includes *Leycesteria* Wallich, *Lonicera* L., and *Symphoricarpos* Duhamel. The monophyly of Caprifolieae has been supported in analyses of morphological characters (Donoghue, 1983; Judd et al., 1994); specifically, they are united by polytelic inflorescences (see Weberling, 1989) and predominance of flavone flavonoids, and also possibly by 4–5 carpels and berry fruits. Caprifolieae monophyly has also been supported in analyses of chloroplast DNA (cpDNA) sequence data, including *rbc*L (Donoghue et al., 1992; Backlund and Bremer, 1997; M. Donoghue, T. Eriksson, P. A. Reeves, and R. G. Olmstead, submitted manuscript), *ndh*F (Pyck et al., 1999; C. Bell and M. Donoghue, unpubl. data), and *mat* K (Kim et al., 1999), as well as by ITS sequences (J. Li and M. Donoghue, unpubl. data). *Heptacodium* Rehder has sometimes also been nested within Caprifolieae (Backlund and Donoghue, 1996), but recent studies of *rbc*L and *ndh*F sequences suggest instead that it is probably the sister group of Caprifolieae (M. Donoghue, T. Eriksson, P. A. Reeves, and R. G. Olmstead, submitted manuscript; Pyck et al., 1999; C. Bell and M. Donoghue, unpubl. data).

Within Caprifolieae, *Triosteum* has been directly united with *Symphoricarpos* in some analyses (Judd et al., 1994; Backlund and Donoghue, 1996). Possible morphological synapomorphies (see Fukuoka, 1972; Wilkinson, 1949) include abortion of one *(Triosteum)* or two *(Symphoricarpos)* carpels, median position of the ovules, ovules supplied by compound vascular bundles, and drupe fruits with mealy-textured mesocarp tissue ("dry drupe," sensu Judd et al., 1994). However, support for relationships within Caprifolieae has been weak, and in other analyses either the relationships are unresolved or alternative arrangements are weakly supported (M. Donoghue, T. Eriksson, P. A. Reeves, and R. G. Olmstead, submitted manuscript). Furthermore, sampling within *Lonicera* has been insufficient to allow rejection of the possibility that *Triostem* and/or *Symphoricarpos* are nested within it. For purposes of rooting *Triosteum*, we included ITS and *waxy* sequences from representative species of *Leycesteria*, *Lonicera*, and *Symphoricarpos*.

Materials and Methods

Terminal Taxa

Table 1 lists the taxa used in this analysis and voucher specimen information. Accessions of all six known species of *Triosteum* from Asia and North America were included, along with two species of *Leycesteria;* three species of *Lonicera*, representing both subgenera, *Lonicera* and *Caprifolium* (Miller) Dippel; and two species of *Symphoricarpos*. Within *Triosteum* we included three accessions each of *T. himalayanum* and *T. pinnatifidum* from different geographic localities. *Triosteum himalayanum* is recognized by its red fruits and entire leaves, and *T. pinnatifidum* by its white fruits and pinnately dissected leaves. We also included an unusual plant from Gongga Shan in Sichuan Province, China, which seemed to combine features of both species, having white fruits like those of *T. pinnatifidum* and entire leaves like *T. himalayanum*. This specimen, labeled "*T.* sp.," was also a very large plant, with much bigger leaves than either of the two species, and could possibly be of hybrid origin.

TABLE 1. Plant material used in this study.

TAXON[1]	LOCALITY[2]	GENBANK	VOUCHER[3]
Triosteum			
T. pinnatifidum a	China: Sichuan Prov., Luhuo Xian	ITS: AF265284 *Waxy*: AF277635	*Boufford et al. 27772*
T. pinnatifidum b	China: Sichuan Prov., Luhuo Xian	ITS: AF265285 *Waxy*: AF277636	*Boufford et al. 27739*
T. pinnatifidum c	Japan: Yamanashi Pref., Mt. Kuchigata	ITS: AF265283 *Waxy*: AF277634	*Shimizu s.n.*
T. himalayanum a	China: Sichuan Prov., Zheduo Tan	ITS: AF265286 *Waxy*: AF277637	*Boufford et al. 27559*
T. himalayanum b	China: Sichuan Prov., Ertaizhi	ITS: AF265288 *Waxy*: AF277639	*Boufford et al. 27525*
T. himalayanum c	China: Sichuan Prov., Xiangcheng	ITS: AF265289 *Waxy*: AF277640	*Boufford et al. 28694*
T. sp.	China: Sichuan Prov., Gongga Shan	ITS: AF265287 *Waxy*: AF277638	*Boufford et al. 27350*
T. aurantiacum	U.S.A: Ohio, Athens Co.	ITS: AF265290 *Waxy*: AF277641	*Walters et al. s.n.* (BHO)
T. perfoliatum	U.S.A: Ohio, Ottowa Co.	ITS: AF265291 *Waxy*: AF277642	*Cantino 1408* (BHO)
T. angustifolium	U.S.A: Ohio, Athens Co.	ITS: AF265292 *Waxy*: AF277643	*Cantino 1407* (BHO)
T. sinuatum	Japan : Nagano, Sugadaira Heights	ITS: AF265293 *Waxy*: AF277644	*Hayashi s.n.*
Symphoricarpos			
S. albus	Cult. AA	ITS: AF265282 *Waxy*: AF277633	*Gilsdorf & Warren 429*
S. orbiculatus	Cult. AA	ITS: AF265281 *Waxy*: AF277632	*Gilsdorf & Warren 431*
Lonicera			
L. involucrata	Cult. AA	ITS: AF265278 *Waxy*: AF277629	*Chapin 104*
L. sempervirens	Cult. AA	ITS: AF265280 *Waxy*: AF277631	*Gould 160* (TEX)
L. caerulea var. *dependens*	Cult. AA	ITS: AF265279 *Waxy*: AF277630	1010 –86-B[4]
Leycesteria			
L. crocothyrsos	Cult. Edinburgh; orig. Assam	ITS: AF265277 *Waxy*: AF277628	Cult. plants of RBG Edinburgh C1123
L. formosa	China: Yunnan Prov., Lijiang	ITS: AF265276 *Waxy*: AF277627	*Boufford et al. 29341*

[1] All leaves were collected from living plants and stored in silica gel, except for *Triosteum sinuatum*, for which dried leaf tissue was taken from a herbarium specimen. Letters following species names (a, b, c) refer to different individuals of the same species.

[2] "Cult. AA" refers to plants cultivated at the Arnold Arboretum, Jamaica Plain, Massachusetts.

[3] Herbarium specimens are in A unless otherwise indicated.

[4] Accession number for living plant in the Arnold Arboretum.

DNA Extraction

Leaf material from most taxa was collected in the field or from cultivated plants growing at the Arnold Arboretum and was stored in silica gel dessicant. Total DNA was isolated from all accessions using a mini-prep CTAB/chloroform extraction procedure (incubating ground tissue in a 2X CTAB, 4% PVP buffer), followed by glass bead purification using the GeneClean II Kit (Bio 101).

Amplification and Sequencing

The internal transcribed spacer (ITS) region of nuclear ribosomal DNA was amplified using primers "ITS7A" (provided by A. Plovanrich to J. Panero, unpublished data) and ITS4 (White et al., 1990) in the presence of 10% dimethyl sulfoxide (DMSO). The PCR product was gel-purified in 1% agarose and extracted with the QIAquick Gel Extraction Kit (Qiagen). The purified PCR product was sequenced directly, using either the BigDye (ABI) or the Sequenase (Amersham) cycle-sequencing kits, and run on an ABI model 377 automated DNA sequencing system. Sequencing primers were ITS2, ITS3, ITS4, and ITS5 or ITS1 of White et al. (1990). This allowed the entire region to be sequenced in both directions except close to the priming sites.

A portion of the *waxy* gene was PCR-amplified using primers *GBSS*F2 and *GBSS*R2 (Li and Donoghue, 1999). The amplified region of the gene included the 5' end of exon 9; introns 9, 10, and 11; exons 10 and 11; and the 3' end of exon 12. PCR purification and sequencing followed the procedures described above. The same primers used for amplification were used for sequencing. The two sequenced strands included, on average, 474 bases of overlap. Although we saw no evidence of multiple copies of *waxy* in Lonicereae (e.g., additional bands), we note that direct sequencing did sometimes yield unreadable sequences. Only clearly readable sequences were included in our phylogenetic analyses.

Phylogenetic Analyses

Sequences were edited using the Sequencher 3.0 program (Gene Codes Corp.) and aligned in Clustal (Thompson et al., 1994). For the ITS region, the ITS1 and ITS2 spacer regions and the intervening 5.8S gene were included in the analysis. For *waxy*, only the region from intron 9 to intron 11, including exons 10 and 11, was used. Alignment gaps were scored as missing data. All sequences are available in Genbank (accession numbers for ITS: AF265276–AF265293; for *waxy*: AF277627–AF277644), and complete data matrices can be obtained from the senior author.

Parsimony analyses (with all characters weighted equally) were carried out using the branch-and-bound search algorithm in PAUP* (beta vers. 4.0b2; Swofford, 1999). Bootstrap values (Felsenstein, 1985) for each branch were estimated on the basis of 100 replicates, each analyzed using a heuristic search with simple addition sequence, TBR branch swapping, and maxtrees set to 500. All additional analyses were carried out using PAUP*, including the partition-homogeneity, or incongruence length difference (ILD), test (Farris et al., 1994), using 100 random repartitions of the data, constraint tests, and Kishino-Hasegawa tests (Kishino and Hasegawa, 1989). Maximum parsimony trees were generated independently for each data set, followed by a combined analysis. On the basis of previous morphological and molecular studies, trees were rooted along the *Leycesteria* branch (M. Donoghue, T. Eriksson, P. A. Reeves, and R. G. Olmstead, submitted manuscript; see above).

Results

Data Characteristics

Individual ITS sequences within *Triosteum* ranged in length from 612 to 617 base pairs (bp), whereas sequences from the other taxa ranged from 608 to 616 bp. The aligned length of the ITS sequences was 626 bp: alignment was unproblematical, and no regions were excluded from the phylogenetic analysis. The ITS region showed low levels of variation. Pairwise differences, ignoring gaps, ranged from 0.8% to 7.8% among *Triosteum* species and among all taxa. There were a total of 54 parsimony-informative ITS characters.

For the region of *waxy* included in our analyses, individual *Triosteum* sequences ranged in length from 766 to 777 bp; among all taxa, sequences ranged from 762 to 809 bp. The aligned sequences were 896 bp in length. Pairwise differences, ignoring gaps, ranged from 0% to 11.4% when contrasting all taxa, and from 0% to 4.1% within *Triosteum*. The most variable region was intron 10 (287–490 bp), containing 19 informative changes. In total, there were 63 parsimony-informative *waxy* characters.

Phylogenetic Analyses

The ITS analysis resulted in 65 most-parsimonious trees of 140 steps (CI [consistency index], minus uninformative characters = 0.756; RI [retention index] = 0.854), excluding 160 polytomous trees for which compatible resolved trees existed. In the strict consensus tree (Fig. 1A), *Symphoricarpos* is monophyletic, but *Lonicera* is paraphyletic, with *L. sempervirens* (subgenus *Caprifolium*) more closely related to *Triosteum*. Within *Triosteum*, *T. sinuatum* is sister to a clade containing the other two Asian species plus the North American species (supported by a boostrap value of 82%). Within the Asia–North America clade, the North American species form a clade (100%), within which *T. aurantiacum* and *T. angustifolium* are sister species. The Chinese species, *T. himalayanum* and *T. pinnatifidum*, do not form a clade. Instead, *T. himalayanum* appears to be paraphyletic with respect to a *T. pinnatifidum*–North American clade (25%). The Gongga Shan specimen, *T.* sp., is united with the *T. pinnatifidum* accessions in an unresolved polytomy.

The *waxy* analysis resulted in 18 most-parsimonious trees of 210 steps (CI, minus uninformative characters = 0.793; RI = 0.862), and only 4 trees after filtering to remove polytomous trees for which compatible resolved trees existed. In the strict consensus tree (Fig. 1B), *Triosteum*, *Symphoricarpos*, and *Lonicera* are each monophyletic, but relationships among them are unresolved. Within *Triosteum*, the North American species form a clade (99% bootstrap support), within which relationships are unresolved. In contrast to the ITS analysis, all of the Asian species, including *T. sinuatum*, form a separate clade, though with weak bootstrap support (42%). Also, the monophyly of the Chinese species is strongly supported by *waxy* sequences (97%). *Triosteum pinnatifidum* accessions are again monophyletic; relationships of the *T. himalayanum* accessions are not fully resolved, and they may be paraphyletic with *T. pinnatifidum* nested within. In this analysis, *T.* sp. is seen to be the sister group of the *T. pinnatifidum* accessions.

The ILD test returned a p value of 0.10, meaning that the summed tree lengths from the original data sets were not significantly lower than the summed tree lengths from the random partitions of the combined data sets. This implies that the data sets do not contain significantly conflicting phylogenetic signals. A combined ITS plus *waxy* analysis resulted in three most-parsimonious trees of length 355 (CI, minus uninformative characters = 0.754; RI = 0.840). The strict consensus tree is shown in Fig. 1C, and a single phylogram is shown in Fig. 1D to illustrate branch lengths. *Triosteum*, *Symphoricarpos*, and *Lonicera* are each seen to be monophyletic (with rather high bootstrap support), but relationships among the three clades are still unresolved. *Triosteum sinuatum* is positioned at the base of *Triosteum*, as in the ITS analysis, with the clade containing the remaining species supported by a bootstrap value of 87%. There is also high bootstrap support for the monophyly of the North American species (100%) and the clade containing the Chinese species (98%).

Within the Chinese clade, both *T. pinnatifidum* and *T. himalayanum* accessions are monophyletic. Here it is noteworthy that the Japanese accession of *T. pinnatifidum* (labeled "c"; see Table 1) is nested within the *T. pinnatifidum* clade with the "a" and "b" accessions from China. As for the placement of the unusual, possibly hybrid, plant from Gongga Shan, in all three analyses it appears to be most closely related to *T. pinnatifidum*, with which it shares white fruits, and not to *T. himalayanum*, with which it shares entire leaves. In the *waxy* and combined analyses, it is the sister group of *T. pinnatifidum*, and in the ITS analysis it appears in an unresolved clade with all *T. pinnatifidum* accessions.

Analyses were also constrained to find only trees containing a clade consisting of the North American species plus *T. sinuatum*. When the ITS data were run with this constraint, 40 trees of length 142 were obtained, that is, only 2 steps longer than the most-parsimonious unconstrained trees. When the *waxy* data were run with this constraint, 54 trees of length 211 were found, only 1 step longer than the unconstrained trees. When the combined data were constrained, 3 trees of length 358 were obtained; 3 steps longer than when unconstrained. The Kishino-Hasegawa test using this constraint tree did not return significant results (ITS only, p = 0.41; *waxy* only, p = 0.56; combined data, p = 0.18), implying that trees containing a *T. sinuatum*–North America clade are not strongly rejected by our data.

Discussion

Phylogenetic Relationships

Both ITS and *waxy* data strongly support the

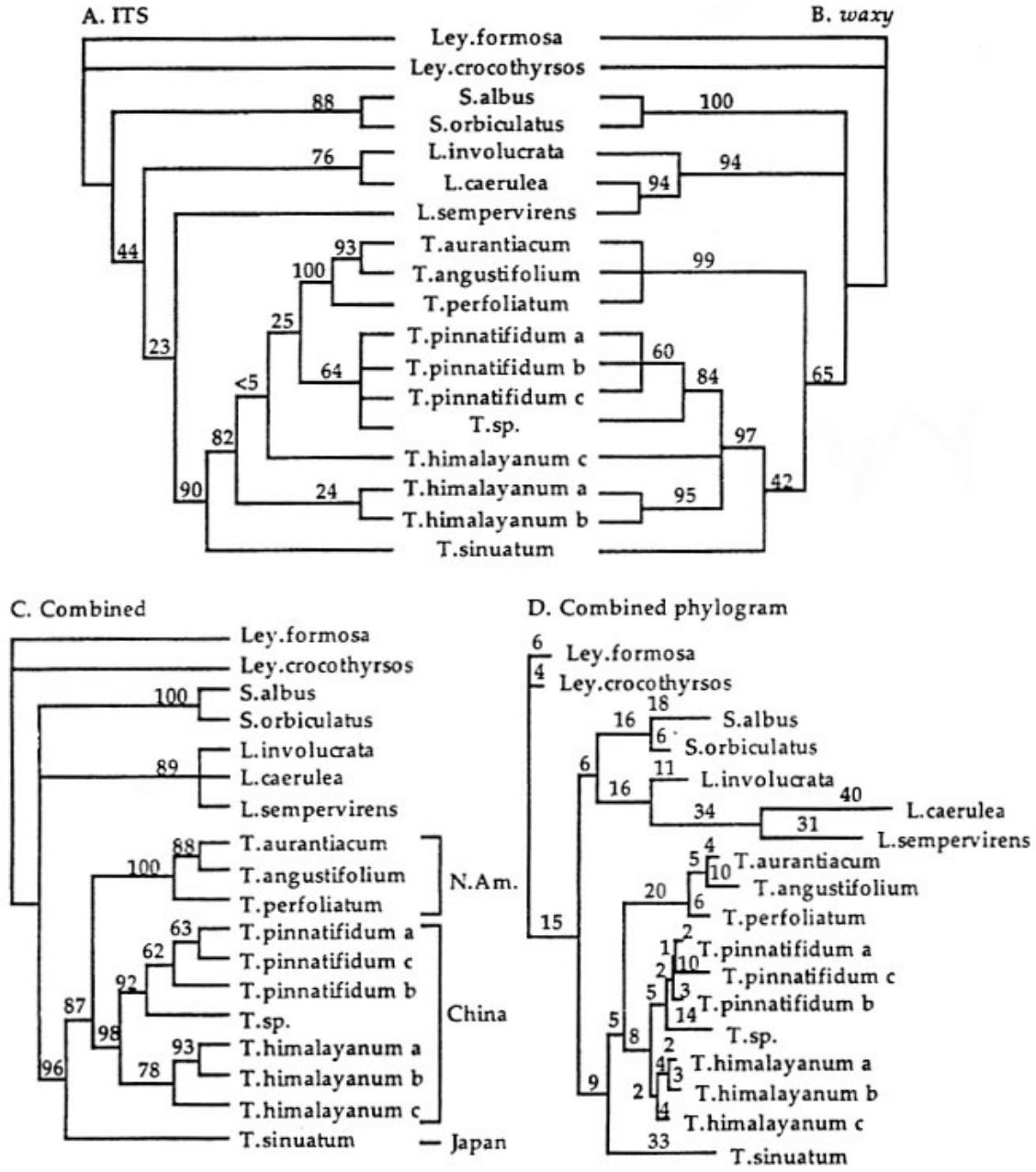

FIGURE 1. A–C. the strict consensus of equally parsimonious trees obtained from analyses of *Triosteum* and other Caprifolieae. with bootstrap values shown above branches. A, ITS data alone; B, *waxy* data alone; C. combined ITS and *waxy* data. D, combined data analysis phylogram (one of three shortest trees) showing branch lengths. Taxon names correspond to those in Table 1.

monophyly of the North American species of *Triosteum*. ITS sequences weakly suggest that the Chinese species are paraphyletic, whereas *waxy* data strongly support the monophyly of the Chinese species. ITS finds a clade containing the Chinese and North American species, with *T. sinuatum* at the base. In contrast, *waxy* weakly supports a monophyletic Asian clade, including *T. sinuatum* at the base. Neither data set, however, groups *T. sinuatum* with the North American species, as suggested by morphology.

Differences between the ITS and *waxy* trees do not represent strong conflicts, and probably are due to sampling error. Neither data set, on its own, is able to resolve all relationships with confidence, and apparently conflicting nodes are only weakly supported by one of the data sets. Most importantly, in the *waxy* tree, the weakest node is that uniting *T. sinuatum* and the Chinese species (with 42% bootstrap support).

Our combined analysis shows complementarity and better support throughout. For example, the monophyly of the Chinese species, which is largely supported by *waxy* sequences, rises to a bootstrap value of 98%. The North America–China clade has 82% bootstrap support in the ITS analysis but 87% in the combined analysis. In addition, the sister relationship of "*T.* sp." from Gongga Shan with *T. pinnatifidum* rises from 64% in the ITS analysis to 84% in the *waxy* analysis to 92% in the combined analysis. We believe that the combined analysis provides the best current estimate of phylogenetic relationships in this group. However, as our Kashino-Hasegawa tests on constrained trees demonstrate, the present data cannot strongly reject several alternative phylogenetic hypotheses. Morphological and biogeographic conclusions based on these results must therefore be treated cautiously and may change as additional data become available.

Morphological Evolution

The morphological characters shared by *T. sinuatum* and the North American species are axillary inflorescences and long calyx lobes. Neither the ITS nor the *waxy* analyses, nor the two combined, supports a clade including just these taxa. Considering just the *Triosteum* clade in the combined tree, the most-parsimonious reconstruction of character-state changes implies that the ancestor of the group had axillary inflorescences and long calyx lobes and that the change to terminal inflorescences and to short calyx lobes both occurred along the branch leading to the Chinese species. For both characters, this picture is not rendered much clearer when outgroups are considered, as these show considerable variation.

Both inflorescence conditions are found in *Symphoricarpos* (varying both within and between species) and *Lonicera* (differentiating the two subgenera, *Lonicera* and *Caprifolium*); and *Leycesteria*, with large inflorescence bracts, might be interpreted as showing an intermediate condition. Taken together, these observations are at least consistent with the view that axillary inflorescences were ancestral for *Triosteum*. A more certain assessment will require further resolution of relationships within and among the genera of Caprifolieae, as well as studies of development.

Regarding calyx lobes, *Lonicera* and *Symphoricarpos* both have small lobes, whereas larger lobes are found in most *Leycesteria*. The persistent calyx lobes of *L. crocothyrsos* are particularly similar to those of *T. sinuatum* and the North American species in shape, size, and appearance in fruit. These observations are consistent with the interpretation that calyx lobes were reduced in a *Lonicera-Symphoricarpos* line and then independently within *Triosteum*. Confident resolution of this issue will again require better understanding of relationships in Caprifolieae as a whole.

Fruit color also varies dramatically within Caprifolieae, especially within *Lonicera*, whose species have fruits ranging in color from orange to red, yellow, purple, blue, and black, and which also vary in opacity, translucence, and bloom. *Leycesteria* fruits may be translucent yellow-green, or changing with maturity from green to maroon to purple-black. Most *Symphoricarpos* species display purple-red or white fruits, with the one Chinese species having purple-black fruits. In *Triosteum*, *T. sinuatum* is said to have a greenish fruit (Hara, 1983), which differs from all others. In North America, *T. aurantiacum* and *T. angustifolium* both have shiny orange-red fruits, whereas *T. perfoliatum* fruits are dull yellowish-orange (P. Cantino, pers. comm.). Among the Chinese species, *T. himalayanum* has shiny red fruits, and *T. pinnatifidum* has white. Reddish-colored fruits may be ancestral for the Chinese–North America clade, and white color derived in *T. pinnatifidum*. However, the level of homoplasy is so high that we are not confident in this assessment.

The placement of *T.* sp. "between" *T. pinnatifidum* and *T. himalayanum* could be viewed as supporting a hybrid origin (e.g., see McDade, 1992). However, given that this population was not found near either of the other species, and given the very large size of the plants, it may also be a distinct species. If so, its position in the tree implies that white fruits evolved first, in the ancestor of the clade including *T.* sp. and *T. pinnatifidum*, followed by evolution of the pinnately dissected leaves characteristic of *T. pinnatifidum*. Chloroplast DNA data would be especially useful in clarifying this situation.

In summary, our results suggest that the first *Triosteum* species had axillary inflorescences and large calyx lobes, similar to those found in *T. sinuatum* and the North American species. However, given the virtual certainty that these characters have been homoplastic in Caprifolieae, and in view of uncertainty about relationships within the group as a whole, we remain somewhat skeptical of this conclusion. If this view is correct, however, then it appears that several obvious morphological character changes occurred along a branch of the *Triosteum* tree that shows rather few molecular character changes (see Fig 1D). This is of interest in view of the positive correlation between morphological and molecular change seen in some other groups (see Omland, 1994, on ducks).

Biogeography

Our combined analysis does not support a direct phylogenetic connection between the Japanese species, *T. sinuatum*, and the three North American species. Instead, it links a North American clade with a Chinese clade, to the exclusion of *T. sinuatum*. Therefore, *Triosteum* does not appear to correspond to a biogeographic pattern found in several other groups. In *Viburnum* sect. *Pseudotinus*, the Japanese species, *V. furcatum* Blume, is strongly united with the eastern North American species, *V. lantanoides* Michx., to the exclusion of two Chinese species (J. Li and M. Donoghue, unpubl. data). Likewise, in *Hamamelis*, the Japanese species, *H. japonica* Sieb. & Zucc., is most closely related to a New World clade of three species, to the exclusion of the Chinese species, *H. mollis* Oliv. (Wen and Shi, 1999; Li et al., 2000). Other groups show biogeographic patterns broadly consistent with this one but either lack resolution or lack species in one of the areas (e.g., *Aralia* L.; Wen et al., 1998; also see Xiang et al., 1998).

The noncorrespondence of *Triosteum* with this pattern could be explained by invoking dispersal and or lineage sorting among areas (in *Triosteum* or in the other groups). Alternatively, it may mean that there are other general biogeographic patterns involving the same areas but reflecting different vicariance events, perhaps in different time periods. Are there other groups in which, like *Triosteum*, the Chinese and North American species are united to the exlusion of Japanese species? *Pachysandra* Michx. (Buxaceae; see Boufford and Xiang, 1992) and *Diphylleia* Michx. (Berberidaceae; see Ying et al., 1984) may provide other examples of this pattern, but phylogenetic analyses, including molecular data, are needed for a critical test.

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