PERKINSIODENDRON, A NEW GENUS IN THE STYRACACEAE BASED ON MORPHOLOGY AND DNA SEQUENCES

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ABSTRACT

Halesia (Styracaceae) has been considered to comprise three species, two from the eastern United States (*H. carolina*, *H. diptera*), and one from China (*H. macgregorii*). In a prior phylogenetic study of the Styracaceae, analysis of morphological data yielded a clade comprising the three species whereas molecular data (nuclear ITS and plastid *rbcL* and *trnL-trnF*) yielded a placement of *H. macgregorii* as sister to *Rehderodendron*, with the two North American species of *Halesia* placed outside of this clade. We further investigated the phylogenetic placement of *H. macgregorii* relative to other genera in the family with an expanded molecular data set. The results corroborate prior analyses placing *H. macgregorii* with *Rehderodendron* rather than with the North American species of *Halesia*. On this basis, combined with an assessment of morphological character variation among *H. macgregorii*, the other (North American) species of *Halesia*, and *Rehderodendron*, we erect **Perkinsiodendron** as a new genus in the Styracaceae to accommodate *H. macgregorii* and form the new combination **P. macgregorii.** We also provide a lectotype for the species. This change has implications for biogeographical analyses based on generic circumscriptions.

KEY WORDS: China, *Halesia*, new genus, *Perkinsiodendron*, *Rehderodendron*, Styracaceae

RESUMEN

Se ha considerado que *Halesia* (Styracaceae) comprende tres especies, dos del este de los Estados Unidos (*H. carolina*, *H. diptera*), y una de China (*H. macgregorii*). En un estudio filogenético anterior de las Styracaceae, el análisis de datos morfológicos al dio un clado que incluye las tres especies mientras que los datos moleculares (ITS nuclear and *rbcL* and *trnL-trnF* plastídicos) dieron una localización de *H. macgregorii* como hermana de *Rehderodendron*, con las dos especies norteamericanas de *Halesia* situadas fuera de este clado. Investigamos además la situación filogenética de *H. macgregorii* con relación a otros géneros de la familia con un juego de datos moleculares expandido. Los resultados corroboraron los análisis previos colocando a *H. macgregorii* con *Rehderodendron* en vez de unirlo a las especies norteamericanas de *Halesia*. Sobre esta base, combinada con una valoración de la variación de los caracteres morfológicos entre *H. macgregorii*, las otras especies (norteamericanas) de *Halesia*, y *Rehderodendron*, erigimos **Perkinsiodendron** como género nuevo en las Styracaceae para acomodar a *H. macgregorii* y hacemos la nueva combinación **P. macgregorii.** También aportamos un lectotipo para la especie. Este cambio tiene implicaciones para los análisis biogeográficos basados en circumscripciones genéricas.

INTRODUCTION

Halesia J. Ellis ex L. (Styracaceae) is currently considered to comprise three species, with *Halesia carolina* L. and *H. diptera* J. Ellis distributed in eastern North America, and *H. macgregorii* Chun in southeastern China. The genus has been considered to be distinguishable from other genera of the Styracaceae by the combination of four sepals and petals (versus five or more), and winged fruit (versus unwinged or at most ribbed fruit; Fritsch 2004). The three species are easily distinguished from each other by several characters, most notably a two-winged fruit in *H. diptera* (versus four-winged), a shallowly parted corolla in *H. carolina* (versus deeply parted), and stamens in two series of unequal length in *H. macgregorii* (versus stamens of ± equal length).

The most comprehensive phylogenetic study of the Styracaceae, in both morphological and DNA sequence data as well as taxon sampling, is that of Fritsch et al. (2001). In that study, *Halesia* was recovered as

monophyletic with morphological data. Two characters, i.e., predominant number of petals = four, and fruit four-winged (with a later change to the two-winged state in *H. diptera*) supported the clade. In contrast, parsimony analysis of the DNA sequence data, comprising the nuclear ribosomal internal transcribed spacer (ITS) region, the plastid *rbcL* gene, and the plastid *trnL-trnF* intron/spacer region, yielded a phylogenetic placement of *H. macgregorii* as sister to *Rehderodendron* Hu; the North American species of *Halesia* group in various places outside of this clade, depending on the gene or gene combination employed in the analysis. *Halesia macgregorii* grouped most strongly with *Rehderodendron* in the *trnL-trnF* analysis, with a bootstrap (BS) value of 93; in the ITS analysis the clade was supported with BS < 50, and in the combined ITS/*rbcL/trnL-trnLF* analysis it was supported with BS = 88. The two North American species tended to group together in the various molecular analyses, but only with the additional species of *Pterostyrax hispidus* Siebold & Zucc. also grouping in the clade, and only with weak support (BS < 50, 51, or 64, depending on the analysis). A brief discussion of the morphological characters uniting *Halesia*, as well as character variation within the genus, was presented.

Although the study of Fritsch et al. (2001) provided the first evidence of non-monophyly for *Halesia*, the relatively few genes sampled and extensive missing data for some taxa limited the conclusions that could be drawn. Here we increase the number of genes employed to five, i.e , nrITS and four plastid genes, and generate a more complete combined data set, with the aim of increasing phylogenetic resolution and bootstrap support among the samples of *Halesia* to better assess the phylogenetic status of the genus. We also assess the morphological basis for *Halesia* more critically than in the previous phylogenetic study. Based on the overall evidence, a new genus is erected to accommodate *H. macgregorii*.

MATERIALS AND METHODS

Eighteen samples of the Styracaceae from eight genera and 16 species were sampled for DNA sequencing (Appendix 1). The ingroup comprised the genera *Changiostyrax* Tao Chen (1/1 species sampled), *Halesia* (3/3), *Melliodendron* Hand.-Mazz. (1/1), *Rehderodendron* (3/5), *Pterostyrax* Sieb. & Zucc. (3/4), and *Sinojackia* Hu (3/7), which together formed a strongly supported clade in a prior molecular phylogenetic study of the Styracaceae (BS = 94; Fritsch et al. 2001). That clade (henceforth referred to as the 'expanded fruit' clade) was also supported by the morphological synapomorphies of bud scales on fertile shoots present, hypanthium adnate to the ovary wall through the entire length of the ovary wall, and hypanthium notably elongated during fruit development (Fritsch et al. 2001). DNA material of *Parastyrax* W.W. Sm., a genus placed in this clade with morphological data (Fritsch et al. 2001), was not available. The sample included three accessions of *H. macgregorii* to increase confidence in the phylogenetic placement of this species. Representative species of the genera *Alniphyllum* Matsum. [*A. fortunei* (Hemsl.) Makino] and *Bruinsmia* Boerl. & Koord. (*B. styracoides* Boerl. & Koord.) formed the strongly supported (BS = 100) sister clade of the above clade in a prior molecular analysis (Fritsch et al. 2001), a placement supported by the morphological synapomorphy of the articulated distal portion of the pedicel (Fritsch et al. 2001); the same representatives were thus employed here as the outgroup.

Total genomic DNA was extracted from fresh leaf material with the DNeasy Plant Mini Kit (Qiagen, Inc., Valencia, California) as per the manufacturer's protocols. Standard PCR techniques were used to amplify all targeted regions (Dieffenbach & Dveksler 2003) except that HotStart-It *Taq* polymerase (Affymetrix, Santa Clara, California) was used for amplifications. The four plastid regions used were *ndhF* (Olmstead & Sweere 1994), *trnL*$^{(UAA)}$*-trnL*$^{(UAA)}$*-trnF*$^{(GAA)}$ (Taberlet et al. 1991), *trnT*$^{(UGU)}$*-trnL*$^{(UAA)}$ (Taberlet et al. 1991), and *trnS*$^{(GCU)}$*-trnG*$^{(UUC)}$*-trnG*$^{(UUC)}$ (Shaw et al. 2005, 2007); these regions are henceforth referred to as *ndhF*, *trnL-trnF*, *trnT-trnL*, and *trnS-trnG*, respectively. Amplification and direct-sequencing were performed with all primers from Shaw et al. (2007) for *trnS-trnG*; Taberlet et al. (1991) for *trnL-trnF and trnT-trnL*; Olmstead & Sweere (1994) for *ndhF*; and Swensen et al. (1998) and White et al. (1990) for the ITS region. For *trnS-trnG*, the regions were amplified and direct-sequenced with primers trnG$^{(UUC)}$*and trnS$^{(GCU)}$* from Shaw et al. (2007) and primers 5'trnG2G and 5'trnG2S from Shaw et al. (2005). The phylogenetic utility of these plastid regions at low taxonomic levels has been well established (Shaw et al. 2005, 2007), and Fritsch et al. (2001) have demonstrated the phylogenetic utility of the ITS region within the Styracaceae. Additional primers were designed beyond

those of Shaw et al. (2005, 2007) and Taberlet et al. (1991) to sequence various noncoding plastid DNA regions of some samples when traditional primers failed. Two primers were designed to sequence both ends of the *trnS-trnG* intergenic spacer and (first primer only) the 5-prime end of the *trnG* intron (571int to trnG-uuc 5′-ATCCTTTACCTCTCAATGACAGAT-3′ and 571int to trnS-gcu 5′-ATCTGTCATTGAGAGGTAAAG-GAT-3′). For *trnT-trnL*, two primers were designed to sequence both ends of the *trnT-trnL* intergenic spacer and (first primer only) the 5-prime end of the *trnL* intron (S-745trnL-F 5′-TCGACCGTTCAAGTATTTCA-3′ and S-277trnT-R 5′-CGATCTAATAATATACTAATAAG-3′). A third primer was designed for sequencing the middle part of the *trnT-trnL* intergenic spacer (S-705trnL-R 5′-TCGTCTTAACTTTCAACTTTACGA-3′).

DNA sequencing, editing, and alignment were performed as in Fritsch et al. (2015). This study generated 67 new DNA sequences, which were deposited in GenBank (Appendix 1). The concatenated alignment was divided into biologically meaningful partitions corresponding to each of the five genic regions, and MrModeltest2 version 2.3 (Nylander 2004) was implemented with PAUP* to estimate substitution models for each partition under the Akaike Information Criterion, with the best-fitting model subsequently applied to each partition for the Bayesian analyses. We employed parsimony, maximum likelihood (ML), and Bayesian inference (BI) analyses to generate phylogenetic trees, with analyses performed as in Fritsch et al. (2015). Parsimony analyses were conducted in PAUP* version 4.0b10 (Swofford 2002), with relative support for individual clades estimated with the bootstrap method (Felsenstein 1985); the ML analysis was conducted with RAxML 7.2.6 (Stamatakis et al. 2008) by employing the General Time Reversible model of nucleotide substitution under the Gamma model of rate heterogeneity (GTRGAMMA) with clade support estimated with ML bootstraps; and the BI analysis was conducted with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) with clade support estimated with posterior probability (PP).

RESULTS

The parsimony, ML, and BI analyses produced similar topologies over all analyses. No strong topological conflicts [i.e., parsimony BS and ML BS > 80; Bayesian PP > 0.90] were observed among analyses based on any one genic region, or between ITS and the combined plastid data; thus, the data were combined into a single overall analysis.

The expanded fruit clade is strongly supported (parsimony BS = 100, ML BS = 100, PP = 1.00; Fig. 1). The three samples of *Halesia macgregorii* form a clade (99, 99, 1.00) that is sister (100, 99, 1.00) to a clade formed by all the *Rehderodendron* samples (< 50, 73, < 0.80). The two species of North American *Halesia* (*H. carolina* and *H. diptera*) form a weakly supported clade with *Pterostyrax hispidus* (58, 53, < 0.80), and the samples of *Sinojackia* all form a clade (100, 100, 1.00). The rest of the topology exhibits generally weak support (i.e., parsimony and ML BS < 50; PP < 0.80).

DISCUSSION

The phylogenetic placement of *Halesia macgregorii* recovered in our study, i.e., as sister to the clade comprising all samples of *Rehderodendron* rather than to the other species of *Halesia*, corroborates the results from a previous molecular study (Fritsch et al. 2001) yielding the same topology but comprising fewer samples of *H. macgregorii* and *Rehderodendron*, and lower overall clade support. Despite the addition of more gene regions relative to the prior study, most of the remaining parts of the topology are poorly supported (Fritsch et al. 2001). Particularly relevant for this study, the clade that includes the two species of North American *Halesia* also includes *Pterostyrax hispidus*, as it did in previous results (Fritsch et al. 2001). This clade is poorly supported and thus no firm conclusions can be drawn, although morphologically the two species of *Halesia* have several shared characters (Fritsch et al. 2001). Whole plastid genome sequencing is currently underway in an attempt to achieve better phylogenetic resolution within the Styracaceae (P.W.F., H.-C. Wang et al , unpubl. data).

In the prior morphological analysis of the Styracaceae (Fritsch et al. 2001) *Halesia* was recovered as monophyletic, with two characters supporting the clade (predominant number of petals = four, and fruit four-winged). Nonetheless, as noted by Fritsch et al. (2001), other morphological characters in *H. macgregorii* are

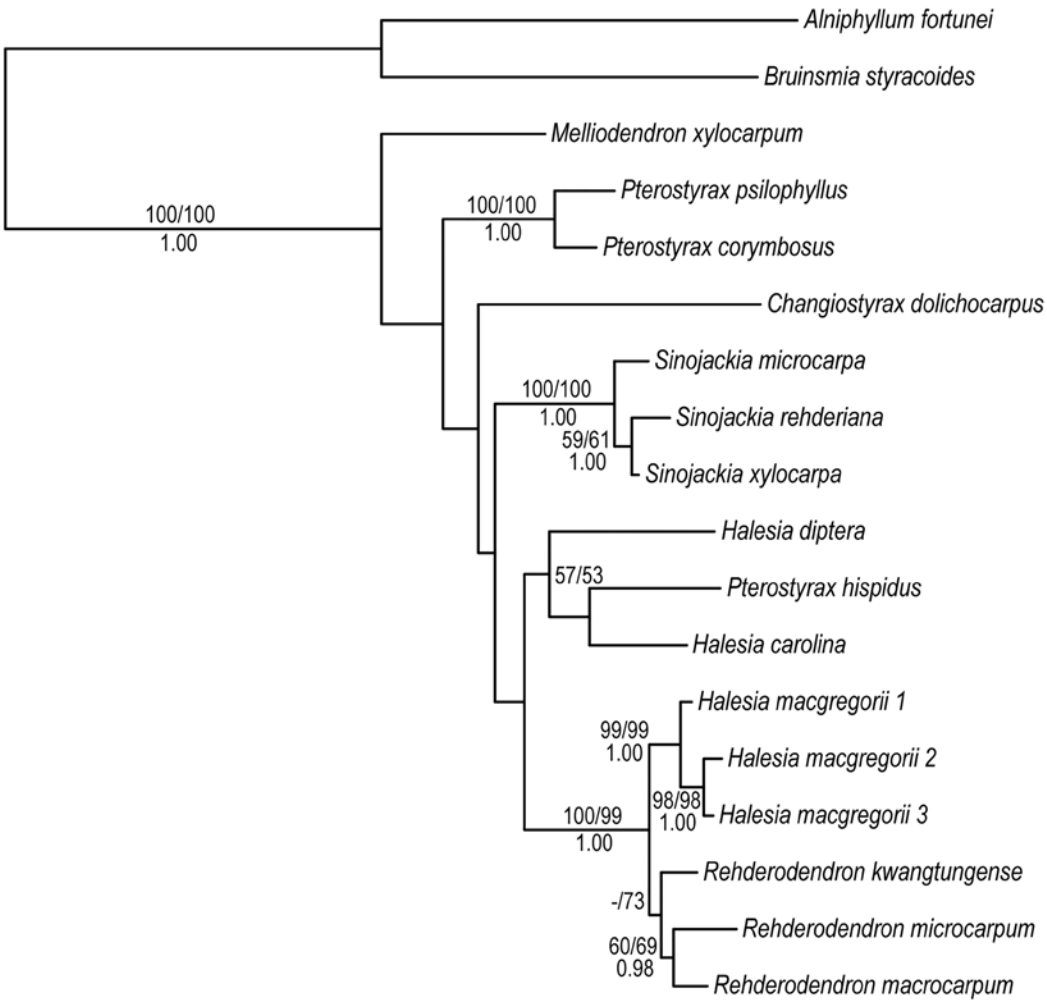

Fig. 1. Best tree recovered from a maximum likelihood phylogenetic analysis of the 'expanded fruit' clade of the Styracaceae and outgroup samples from *Alniphyllum* and *Bruinsmia* based on combined DNA sequence data from four plastid regions and the nuclear ribosomal internal transcribed spacer region. Numbers above branches are parsimony bootstrap values > 50/maximum likelihood values > 50; those below branches are Bayesian posterior probabilities > 0.80.

consistent in its placement as sister to *Rehderodendron* rather than with the North American species of *Halesia*: the species usually has four corolla lobes, but occasionally has five (Hwang & Grimes 1996), as in *Rehderodendron* (versus exclusively four in North American *Halesia*); it has continuous pith, as in all *Rehderodendron* species (versus diaphragmed in North American *Halesia*; Fig. 2); the stamens are of two different lengths in two alternating series, as in all *Rehderodendron* (versus all stamens of equal length in North American *Halesia*; Fig. 2); and the stamens are of definite number (eight in *H. macgregorii* and 10 in *Rehderodendron*), versus an indefinite number in North American *Halesia* (12 to 16 in *H. carolina*, and seven to 10 in *H. diptera*; Table 1).

In light of the sister group relationship between *Halesia macgregorii* and *Rehderodendron* recovered in the molecular analysis, taxonomic revision is warranted to prevent the recognition of paraphyletic genera. Two alternatives are possible: *H. macgregorii* could either be combined with *Rehderodendron* or separated as a distinct genus. The putative synapomorphies for the *H. macgregorii* + *Rehderodendron* clade could provide

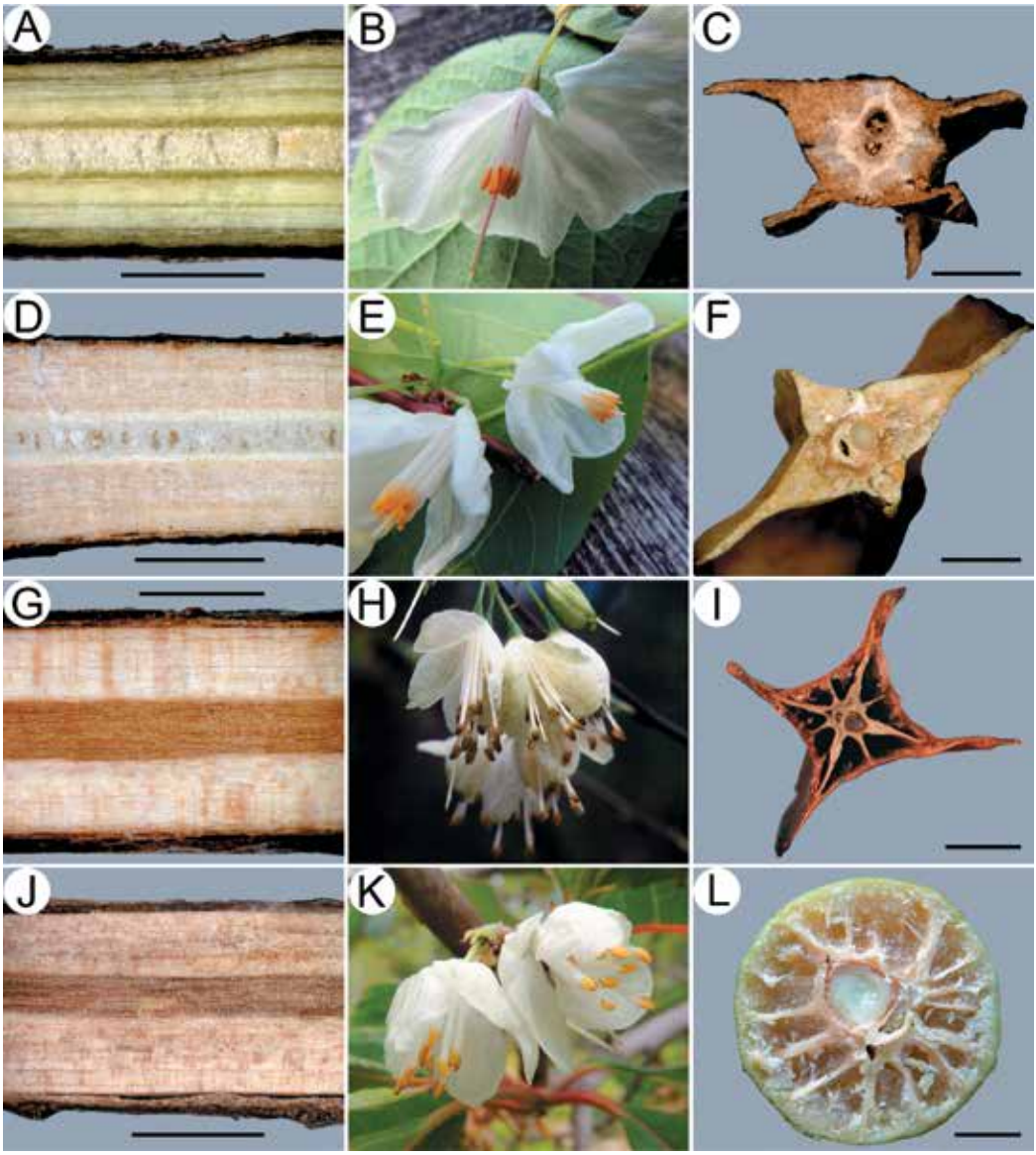

Fig. 2. Branchlets, flowers, and fruit of *Perkinsiodendron* gen. nov., compared with those of *Halesia* and *Rehderodendron*. *Halesia carolina* (A–C), *H. diptera* (D–F), *H. macgregorii* (G–I), and *Rehderodendron* (J–L). **A.** Branchlet in longitudinal section showing spongy chambered pith. **B.** Flower with corolla cut longitudinally and opened to show stamens of nearly equal length. **C.** Fruit in cross section showing the presence of mesocarp. **D.** Branchlet in longitudinal section showing spongy chambered pith. **E.** Flowers showing stamens of nearly equal length. **F.** Fruit in cross section showing the presence of mesocarp. **G.** Branchlet in cross section showing solid pith. **H.** Flowers showing stamens in two series of unequal lengths. **I.** Fruit in cross section showing the absence of mesocarp and presence of 8-ribbed endocarp. **J.** *R. kwangtungense* Chun, branchlet in cross section showing solid pith. **K.** *R. macrocarpum* Hu, flowers showing stamens in two series of unequal lengths. **L.** *R. microcarpum* K.M. Feng, fruit in cross section showing multi-ribbed endocarp with lacunae between the ribs. Scale bars: A, D, G, J = 2 mm; C, F, I, L = 5 mm. [Photos: all by P.W.F., except I by X.S.H. A–C, *P.W. Fritsch 1974* (CAS), D, *E.J. Palmer 12727* (CAS), E, *P.W. Fritsch 1976* (CAS), F, *P.W. Fritsch 1977* (CAS), G, *Longxishan Exp. 2037* (CAS), H, *C.T. Chen & P.W. Fritsch 9704096* (CAS), I, Wuhan Botanical Garden (living collection), J, *C.T. Chen & P.W. Fritsch 9704094* (CAS), K, Quarryhill Botanical Garden 2001.262 (living collection), L, *Gaoligong Shan Biodiversity Survey 32557* (CAS)].

Table 1. Character differences among *Halesia macgregorii*, North American *Halesia* (*H. carolina* and *H. diptera*), and *Rehderodendron*. Data from Chester (1966), Hwang & Grimes (1996), Fritsch (2004, 2009), Fritsch et al. (2001), and P.W.F. personal observations of specimens at CAS and BRIT.

Character	*Halesia macgregorii*	North American *Halesia*	*Rehderodendron*
Branchlet pith	Continuous	Diaphragmed	Continuous
Leaf blades	Glabrous	Pubescent	Glabrous or pubescent
Leaf blade secondary veins on each side of midvein	10 to 24	5 to 9	6 to 13
Pedicel and hypanthium	Glabrous	Pubescent	Pubescent
Sepals	4	4	5
Petals	4 (or 5)	4	5
Corolla	Glabrous	Glabrous *(H. carolina)* or pubescent (*H. diptera*)	Pubescent
Stamens	8	7 to 16	10
Stamen lengths	Unequal in 2 series	± equal	Unequal in 2 series
Stamen filaments	Glabrous	Pubescent	Glabrous or pubescent
Fruit	Winged	Winged	Ribbed
Mesocarp	Absent	Present	Present
Endocarp	Without lacunae	Without lacunae	With lacunae
Seed surface	Areolate	Areolate	Fibrous

justification for combining them into a single genus, but in our view the substantial morphological differences between these two taxa effectively preclude such a change. This is especially clear on the basis of fruit type differences, i.e., fruit winged (versus ribbed), mesocarp absent (versus present), and endocarp without lacunae (versus with lacunae; Table 1; Fig. 2). Similar degrees of fruit differentiation have typically been used to define generic limits throughout the family (Perkins 1907; Chen 1995; Hwang & Grimes 1996). In addition, *H. macgregorii* has 10 to 24 secondary veins on each side of the leaf blade midvein (versus six to 13 in *Rehderodendron*), glabrous pedicels and hypanthia (versus pubescent), four sepals (versus five), glabrous corollas (versus pubescent), eight stamens (versus 10), and areolate seed surfaces (versus fibrous).

In addition to these differences, the alternative of erecting a new genus to accommodate *Halesia macgregorii* is supported by the presence of several characters that uniquely distinguish it from both *Rehderodendron* and the North American species of *Halesia*: 10 to 24 secondary veins on each side of the leaf blade midvein (versus six to 13, and five to nine, respectively), glabrous pedicels and hypanthium (versus pubescent), eight stamens (versus 10 and seven to 16, respectively), and mesocarp absent (versus present; Table 1). Thus, despite the still poorly resolved phylogenetic placement of North American *Halesia* with molecular data, the substantial number of vegetative, floral, and fruit morphological differences among *H. macgregorii*, the other two species of *Halesia*, and all species of *Rehderodendron*, when combined with the robust phylogenetic placement of *H. macgregorii* with *Rehderodendron*, to us warrant the recognition of a new genus. Below we erect *Perkinsiodendron* gen. nov. to accommodate *H. macgregorii*.

This change has potential ramifications for analyses involving the historical biogeography of eastern Asia and eastern North America. Among eastern Asian-eastern North American disjunct genera, more species typically occur in eastern Asia than in North America (Guo & Ricklefs 2000). *Halesia* has been considered to exhibit the reverse pattern, with two species in North America and one in eastern Asia, but our phylogenetic data show this to result from taxonomic artifact. The phylogenetic data are yet unclear as to the precise relationships of the North American species of *Halesia*, but our study demonstrates that there is no direct biogeographic connection with these species and the eastern Asian *H. macgregorii*. Although biogeographic analyses should generally be based on clades, not taxa, the transfer of *H. macgregorii* to *Perkinsiodendron* serves to emphasize the deeper-level biogeographic connections among these taxa than might otherwise be inferred from the retention of *P. macgregorii* in *Halesia*.

TAXONOMIC TREATMENT

Perkinsiodendron P.W. Fritsch, gen. nov. TYPE: *Perkinsiodendron macgregorii* (Chun) P.W. Fritsch (≡ *Halesia macgregorii* Chun).

Haec generi *Halesiae* J. Ellis ex L. simillima, sed ab eo medulla ramuli locellata, lamina glabra, nervis laminae utroque costae latere 10–24, pedicello et hypanthio glabro, staminibus 8 in duabus seriebus inequalibus, filamentis glabris, mesocarpis nullis differt.

Trees, deciduous. **Branchlets** with continuous pith; bud scales present. Fertile shoots lateral only, without fully developed leaves. **Leaves** glabrous, with serrate margin, secondary veins 10–24 on each side of midvein. **Inflorescences** compact axillary racemes, appearing fasciculate, borne at nodes on shoots of previous growing season, 1–7-flowered. Pedicel glabrous, distal portion articulated. **Flowers** opening before leaves. Hypanthium adnate to ovary wall, obconical, 4-ribbed, glabrous. Calyx with open aestivation; sepals tooth-like, 4. Corolla with imbricate aestivation; petals 4(5), connate distinctly beyond their bases, becoming distinct at the same point as their divergence from the androecium, glabrous. Androecium with stamen tube present; stamens 8, adnate to corolla for ca. 2 mm, distinctly unequal in length, in two alternating series; filaments glabrous. Gynoecium 2–4-carpellate; style glabrous, stigma truncate; ovules 4 per carpel in two axial rows, upper apotropous, lower epitropous. **Fruit** indehiscent, 4-winged, with hypanthium notably elongated during development, beak distinct; mesocarp absent; endocarp without lacunae, surface strongly 8-ribbed. Seed to carpel ratio < 1. **Seeds** oblong, terete; surface areolate. One species.

Etymology.—The genus is named in honor of Janet Russell Perkins (1853–1933), the foremost early 20th Century authority on the Styracaceae and the author of the treatment of the family in Das Pflanzenreich (Perkins 1907).

Perkinsiodendron macgregorii (Chun) P.W. Fritsch, comb. nov. BASIONYM: *Halesia macgregorii* Chun, J. Arnold Arbor. 6:144, 217. 1925. TYPE: CHINA. ZHEJIANG: Di-ping, NE of Tai-Shun Hsien, 790 m, 18 Jul 1924, *Ren-Chang Ching, Southeastern Expedition to Chekiang 2132* (LECTOTYPE, designated here: A [2 sheets: barcode 00062559 n.v., internet image https://plants.jstor.org/stable/10.5555/al.ap.specimen.a00062559!, barcode 00062560 n.v., internet image https://plants.jstor.org/stable/10.5555/al.ap.specimen.a00062560!], ISOLECTOTYPES: A barcode 00061968 n.v., internet image https://plants.jstor.org/stable/10.5555/al.ap.specimen.a00061968!, E barcode 00273798 n.v., internet image https://plants.jstor.org/stable/10.5555/al.ap.specimen.e00273798!, US [2: No. 2333839 barcode 00113505 n.v., internet image https://plants.jstor.org/stable/10.5555/al.ap.specimen.us00113505!, No. 1246979 barcode 00513026 n.v., internet image https://plants.jstor.org/stable/10.5555/al.ap.specimen.us00513026!], K barcode 000768026 n.v., internet image https://plants.jstor.org/stable/10.5555/al.ap.specimen.k000768026!).

Halesia macgregorii Chun var. *crenata* Chun, Sunyatsenia 1(4):295. 1934. TYPE: CHINA. GUANGDONG: Yuyuen, Wutung, *S.P. Ko 51908*; Shiuchow, Yiu Shan, Fongtung, *S.P. Ko 51976* (SYNTYPES: IBSC, n.v.).

In the original description of *Halesia macgregorii*, Chun (1925) cited two herbaria as housing the type material: "Specimens...in the Herbarium of Southeastern University, Nanking, and the Herbarium of the Arnold Arboretum." We could not locate material from Southeastern University, but there are three sheets of *R.C. Ching 2132* at the Arnold Arboretum (A). Two comprise a single duplicate, because the label states "2 sheets" on one (62559), and "2nd sheet" on another (62560). On the third sheet (61968), the collection label has a header "National Southeastern University, Nanking, China," and the sheet is stamped with "Presented to the Arnold Arboretum by the trustees of Lingnan University, October 1954." This sheet may be the specimen originally housed at Southeastern University referred to in the protologue, which may have been given to Lingnan University and then to A. Regardless, of the specimens we have seen, the duplicate that comprises the two sheets has the most and best reproductive material and also has what appears to be an original collection label. Furthermore, Edward Chester, in an unpublished dissertation on *Halesia* (Chester 1966), wrote "lectotype" on 62559, and "isotype" on 61968; he indicated on 62559 that the second syntype sheet of this collection (62560) was not sent to him on loan, so he did not see this sheet. To both be consistent with Chester's unpublished lectotype determination and otherwise lectotypify on the best material, we designate the two sheets 62559 and 62560, together comprising the single duplicate at A, as the lectotype.

We have not been able to examine type material of *Halesia macgregorii* var. *crenata* Chun. On the basis of the description it appears to represent merely a large-leaved version of the species with crenate margins. Chester (1966) included this variety in the synonymy of *H. macgregorii*, although he also was not able to examine

any type material of this name. In providing some additional notes and an expanded description and illustration of *H. macgregorii* and other species of the Styracaceae, Chun & Chun (1935) did not comment on the status of the variety.

Distribution.—China (Fujian, Guangdong, Guangxi, Guizhou, Hunan, Jiangxi, Zhejiang (Hwang & Grimes 1996).

Additional Descriptions.—W.Y. Chun, J. Arnold Arbor. 6:144. 1925; Chester (1966:134); S.M. Hwang, Fl. Reipubl. Popularis Sin. 60(2):130. 1987; S.M. Hwang & J. Grimes, Fl. China 15:266. 1996.

Illustrations.—H.H. Hu & W.Y. Chun, Icon. Pl. Sin. 1:44, pl. 44. 1927; H.H. Hu, Bull. Fan Mem. Inst. Biol. 3:320, pl. 16 (64). 1932; S.M. Hwang, Fl. Reipubl. Popularis Sin. 60(2):131, pl. 44 (1–7). 1987; Z.Y. [C.Y.] Wu & P.H. Raven, Fl. China Ill. 15:213, fig. 213 (1–7). 2000.

KEY DISTINGUISHING *PERKINSIODENDRON* FROM *HALESIA* AND *REHDERODENDRON*

1. Branchlet pith diaphragmed; stamens variably 7 to 16, ± equal in length ____ **Halesia**
1. Branchlet pith continuous; stamens consistently 8 or 10, in two unequal series.
   2. Leaf blade secondary veins 6 to 13; pedicel and hypanthium pubescent; stamens 10; fruit ribbed; mesocarp present; endocarp with lacunae; seed surface fibrous ____ **Rehderodendron**
   2. Leaf blade secondary veins 10 to 24; pedicel and hypanthium glabrous; stamens 8; fruit winged; mesocarp absent; endocarp without lacunae; seed surface areolate ____ **Perkinsiodendron**

APPENDIX 1

Sample vouchers (herbarium acronyms in parentheses) with source information and GenBank accession numbers. Accession numbers KU936355 through KU936422 were newly generated.

Taxon, source and collection number (herbarium acronym), GenBank accession numbers for ITS, *trnL-trnF, trnT-trnL,* and *trnS-trnG*

Alniphyllum fortunei (Hemsl.) Makino, Nanyue Arboretum, Hunan, China, *C.T. Chen & P.W. Fritsch 9704086* (CAS), AF396437, AF396161 & AF396162, KU936361, KU936405, KU936379; *Bruinsmia styracoides* Boerl. & Koord., Sabah, Malaysia, *C.H. Cannon 529* (DUKE), AF396438, AF396163 & AF396164, KU936362, KU936406, KU936380; *Changiostyrax dolichocarpa* (C.J. Qi) Tao Chen, Hunan, China, *C.T. Chen & P.W. Fritsch 940005* (IBSC), AF396439, AF396165 & AF396166, KU936363, KU936407, KU936381; *Halesia carolina* L., University of California Botanical Garden, Berkeley, U.S.A., 86.0264, *P.W. Fritsch 1481* (CAS), AF396440, AF396167 & AF396168, KU936364, KU936408, KU936382; *H. diptera* J. Ellis, University of California Botanical Garden, Berkeley, U.S.A., 92.0002, *P.W. Fritsch 1482* (CAS), AF396441, AF396169 & AF396170, KU936365, KU936409, KU936383; *H. macgregorii* Chun Accession 1, Hangzhou Botanical Garden, Zhejiang, China, *C.T. Chen & P.W. Fritsch 9704069* (CAS), KU936355, KU936397 & KU936398, KU936366, KU936410, KU936384; *H. macgregorii* Chun Accession 2, Nanyue Arboretum, Hunan, China, *C.T. Chen & P.W. Fritsch 9704096* (CAS), AF396442, AF396171 & AF396172, KU936367, KU936411, KU936385; *H. macgregorii* Chun Accession 3, Hunan Forest Botanical Garden, Hunan, China, *P.W. Fritsch, s.n.* (no voucher), KU936356, KU936399, KU936368, KU936412, KU936386; *Melliodendron xylocarpum* Hand.-Mazz., Nanyue Arboretum, Hunan, China, *C.T. Chen & P.W. Fritsch 9704087* (CAS), AF396444, AF396177 & AF396178, KU936369, KU936413, KU936387; *Pterostyrax corymbosus* Siebold & Zucc., Hangzhou Botanical Garden, Zhejiang, China, *C.T. Chen & P.W. Fritsch 9704067* (CAS), AF396445, AF396179 & AF396180, KU936370, KU936414, KU936388; *P. hispidus* Siebold & Zucc., University of California Botanical Garden, Berkeley, U.S.A., 90.0671, *R. Shefferson s.n.*, 29 May 2001 (UC), KU936357, KU936400, KU936371, KU936415, KU936389; *P. psilophyllus* Diels ex Perkins, University of California Botanical Garden, Berkeley, U.S.A., 92.1047, *P.W. Fritsch 1483* (CAS), AF396447, KU936401, KU936372, KU936416, KU936390; *Rehderodendron kwangtungense* Chun, Nanyue Arboretum, Hunan, China, *C.T. Chen & P.W. Fritsch 9704094* (CAS), AF396448, AF396185 & AF396186, KU936373, KU936417, KU936391; *R. macrocarpum* Hu, Washington Park Arboretum, Seattle, U.S.A. 603-38, *P.W. Fritsch 1359* (RSA), AF396449, AF396187 & AF396188, KU936375, KU936419, KU936393; *R. microcarpum* K.M. Feng, Yunnan, China, *Gaoligong Shan Biodiversity Survey 32557* (CAS), KU936358, KU936402, KU936374, KU936418, KU936392; *Sinojackia microcarpa* Tao Chen & G.Y. Li, Zhejiang, China, *C.T. Chen & P.W. Fritsch 9704080* (CAS), KU936359, KU936403, KU936377, KU936421, KU936395; *S. rehderiana* Hu, Hangzhou Botanical Garden, Zhejiang, China, *C.T. Chen & P.W. Fritsch 9704079* (CAS), AF396450, AF396189 & AF396190, KU936376, KU936420, KU936394; *S. xylocarpa* Hu, Botanical Garden of Zhejiang Agricultural University, Zhejiang, China, *C.T. Chen & P.W. Fritsch 9704059* (CAS), KU936360, KU936404, KU936378, KU936422, KU936396.

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