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 dio un clado que incluye las tres especies mientras que los datos moleculares (ITS nuclear y 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, erguimos 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 circunscripciones 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)−*trnG(GAA) (Taberlet et al. 1991), *trnT(UAG)−trnL(UAA) (Taberlet et al. 1991), and *trnS(G-CU)−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 Swenson 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(G-CU)* 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′-end of the trnG intron (571int to trnG-ucc 5′-ATCTTTAACCCTCTCAATGACAGAT-3′ and 571int to trnS-gcu 5′-ATCTGTCATTGAGAGGTAAAGGAT-3′). For trnT-trnL, two primers were designed to sequence both ends of the trnT-trnL intergenic spacer and (first primer only) the 5′-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
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

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**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.
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). 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 hypanthia (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.
Perkinsiodendron PW. Fritsch, gen. nov. **Type:** Perkinsiodendron macgregorii (Chun) PW. Fritsch (= Halesia macgregorii Chun).

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


**Halesia macgregorii** Chun var. crenata Chun, Sunyatsenia 1(4):295. 1934. **Type:** CHINA. Guangdong: Yuyuen, Wutung, S.P. Ko 51908; Shuchow, Yu 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 1934.” 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 “ISOETYPE” 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).


**APPENDIX 1**

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

**Key Distinguishing Perkinsiodendron from Halesia and Rehderodendron**

1. Branchlet pith diaphragmed; stamens variably 7 to 16, ± equal in length  
   ________________ Halesia

2. 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

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

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<th>Taxon</th>
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