

## **Not out of the box: phylogeny of the broadly sampled Buxaceae**

Running title: Phylogeny of Buxaceae

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### **Abstract and keywords**

The Buxaceae constitute a morphologically diverse phylogenetic lineage of six genera, which includes about 140 species. The most well-known genera are *Buxus*, *Sarcococca*, and *Pachysandra*. Few species of woody *Styloceras* grow on mid-elevations in the Andes mountains region. *Didymelis*, with three species endemic to Madagascar, and the monotypic *Haptanthus* from Honduras, are the most unusual members of the group. The infra-familial classification of Buxaceae is controversial, and molecular data about many species, especially Old World, is still lacking. We used broad taxonomic sampling and molecular data from four chloroplast markers, and the nuclear ribosomal ITS to estimate their phylogeny. These data provide phylogenetic placements of 50 species and enabled better estimates of boundaries in Buxaceae. We described two subfamilies, two monotypic genera, two *Buxus* subgenera, and one new species of *Didymelis* from Madagascar.

Keywords: Buxaceae, *Haptanthus*, *Didymelis*, *Buxus*, *Sarcococca*, *Pachysandra*, *Styloceras*, phylogeny

## Introduction

Within flowering plants, the boxwood family, Buxaceae Dumort. (Dumortier, 1822), together with *Haptanthus* A. Goldberg & C. Nelson (Goldberg & Nelson, 1989) and *Didymelis* Thouars (Thouars, 1804) form an old (Takahashi et al., 2017), distinct (Worberg et al., 2007; Gutiérrez, 2014), and diverse (about 140 species) taxon. They are distributed almost worldwide (Fig. 1) with high species diversity in Tropical America, Southern Africa / Madagascar, and East Asia (Jarvis, 1989; Köhler, 2004; Köhler, 2007; Köhler, 2009). They have no close relatives; the nearest branch on the phylogenetic tree of angiosperms are East Asian *Trochodendron* and *Tetracentron*, to which they were not even considered to be related before the “molecular era” (Castilho et al., 1999; Takhtajan, 2009).

There are multiple morphological characters which unite the group (Oskolski & al., 2015), for example, cyclocytic or laterocytic stomata, frequently triplinerved leaf venation, racemose inflorescences, frequently dimerous flowers, stamen-sepalum complex in many representatives, and unusual pregnan steroid alkaloids (Hardman, 1987; von Balthazar & Endress, 2002a, b; von Balthazar et al., 2003; Köhler, 2007).

Two anomalous genera of the group have been variously treated as independent families or as part of the Buxaceae. *Haptanthus* was discovered in Atlantida province of Honduras (Goldberg & Nelson, 1989) and segregated as a monotypic family based on its perianth structure (Nelson, 2001; Shipunov, 2003), but the actual taxonomic position has been heavily deliberated (Doust & Stevens, 2005; Goldberg & Alden, 2005). As a result, it was accepted as the only *incertae sedis* genus of flowering plants by Takhtajan (2009) and even mentioned as such in the example 1 of ICN Article 3.1 (“Ex.1. The genus *Haptanthus* Goldberg & C. Nelson (in Syst. Bot. 14: 16. 1989) was originally described without being assigned to a family...”). The *locus classicus* was apparently lost through deforestation, but *Haptanthus* was recently re-discovered (Shipunov & Shipunova, 2010) and also found in more locations. Since then, it has been established *ex situ* in the Lancetilla Botanical Garden, Honduras (Bejarano, 2015). Both morphologically and molecularly, *Haptanthus* has similarities with the “core Buxaceae” as well as with *Didymelis* (Shipunov & Shipunova, 2010; Oskolski & al., 2015).

Geographically, *Didymelis* is close to the South African / Madagascan center of the group diversity, but until the “molecular era”, it was not considered as a relative to the core Buxaceae (Worberg et al., 2007; Takhtajan, 2009). There are three (Köhler, 2007) or two (Madagascar Catalogue, 2018) accepted species but also some unidentified and putatively new material (Madagascar Catalogue, 2018). One of the most striking characters of *Didymelis* is the presence of a monomerous gynoecium (Leandri, 1937).

Among the core genera of the Buxaceae, *Buxus* (Linnaeus, 1753) is the most speciose (ca. 110 species) and well-known (Larson, 1996) group. The infrageneric taxonomy of boxwoods is highly correlated with geography, and American and African species were at first considered as separate genera: the American *Tricera* (Schreber, 1797) and the African *Notobuxus* (Oliver, 1882). Van Tieghem (1897) split African *Buxus* in three genera, *Buxanthus* Tiegh., *Buxella* Tiegh., and *Notobuxus*. Later taxonomic revisions treated these as sections or subgenera, e.g., sect. *Tricera* (Schreb.) Baill. and subg. *Probuxus* Mathou (Baillon, 1859; see also Mathou, 1940). Though *Buxus* is widely distributed and highly diverse in comparison to other genera in the family, the apparently rapid speciation of *Buxus* on Cuba, which has almost 40 endemic boxwood species (Sauget & Liogier, 1974; Köhler, 1998; Köhler, 2004; Köhler, 2006; Gutiérrez, 2014; Köhler, 2014), is remarkable relative to the diversity elsewhere. Despite their overall similarities, there are some substantial differences between these subgroups. For

example, the Old World *Buxus* typically have cortical vascular bundles in each angle of the branchlets, accompanied by fiber strands in the Eurasian taxa (Köhler, 2004; Köhler, 2007), whereas both these characters are absent in New World *Buxus*.

The subfamily *Pachysandroideae* Record & Garratt (or tribe *Pachysandreae* Reveal: Record & Garratt, 1925; Reveal, 2011, 2012) is comprised of three genera: *Pachysandra* Michx., *Sarcococca* Lindl., and *Styloceras* Kunth. The three species of *Pachysandra* (Michaux, 1803) are herbaceous or suffruticose rhizomatous plants (Robbins, 1968), and their disjunct distributions are classic examples of the East Asian — Eastern North American floristic disjunction (Gray, 1846; Raven & Axelrod, 1974). *Pachysandra procumbens* Michx. (“Allegheny spurge”), from North America, and *P. terminalis* Siebold & Zucc. (“kichijiso”) from Japan and China, are common garden plants used extensively as ground covers (Batchelor & Miyabe, 1893; Dirr & Alexander, 1979; Channell & Wood, 1987). *Pachysandra terminalis* differs significantly from the two other species in its terminal inflorescences, strongly spreading rhizomatous habitat, bicarpellate gynoecium, and white baccate fruit. *Pachysandra procumbens* and the Chinese polymorphic *P. axillaris* Franchet have been shown to be sister species in molecular phylogenetic analyses (Jiao & Li, 2009) despite their different appearance and the geographic proximity of the latter to *P. terminalis*.

The species of *Sarcococca* (Lindley, 1826) are small shrubs and understory plants of humid lowland and mountain forests, mostly in southeastern Asia. With their evergreen habit, winter-flowering, and fragrant flowers, the species of *Sarcococca* are generally referred to as “Sweet Box” (Sealy, 1986). Sealy’s (1986) revision was a first step in resolving some of the many taxonomic problems and specific boundaries in *Sarcococca*. Despite his efforts, regional floristic treatments do not recognize the same suite of taxa or the same synonymy (Backer & Bakhuizen van den Brink, 1965; Min & Brückner, 2008) and there is extensive variation in morphology across the distributions of some species. *Sarcococca* is also remarkable with the apparent geographic disjunction of *S. concinna* (Standl.) I.M. Johnston, which occurs in southern Mexico (Oaxaca) and Guatemala (Johnston, 1939). It was first described as a *Buxus* (Standley, 1936; Johnston, 1938), but Sealy (1986) doubted it belonged there or with *Sarcococca* based on its morphology, inflorescence structure, and fruit type.

The Andean *Styloceras* (Kunth, 1824) are small trees or shrubs, and with the exception of *Buxus citrifolia* (Willd.) Spreng. are the only representatives of Buxaceae in South America. *Styloceras* has been treated as a monogeneric family, the Stylocerataceae Takht. ex Reveal & Hoogland (Reveal & Hoogland, 1990), though it is now shown to be embedded within the Buxaceae (von Balthazar & al., 2000). Six species are currently recognized (Gentry & Aymard, 1993; Torrez & Jorgensen, 2010; Ulloa Ulloa & al., 2017), but we believe that the full diversity of this rare group is not yet understood.

The infra-familial classification of Buxaceae is still controversial. Mathou (1940) used two tribes, *Buxeae* Dumort. (with *Buxus* only) and *Pachysandreae* (with the other three genera of core Buxaceae), whereas Takhtajan (2009) essentially raised each genus into its own tribe. Reveal (2012) used two subfamilies, *Pachysandroideae* and *Buxoideae* Beilschm. (Beilschmid, 1833). The recent work on *Buxus* Caribbean taxa (Gutiérrez, 2014) provided a starting point for understanding the evolution of the significant portion of American boxwood species and group as a whole, but data about many other species, especially Old World, is still lacking. There is no recent synthetic classification of the family.

We attempt to provide a comprehensive classification scheme for Buxaceae through high taxonomic sampling that will serve as a framework for future studies in the group.

## Materials and Methods

Our sampling protocol, due to the broad geographic distribution of the Buxaceae and rarity of many taxa, used herbarium material wherever possible for DNA extractions. We extracted DNA from 286 samples: 271 samples from herbaria and 15 from our collections. Herbarium tissue samples were obtained from numerous herbaria (B, BO, BRIT, CAS, F, HUH, IBSC, JEPS, NBG, NY, PE, PRE, SAM, SP, SPF, TI, US, and USM) with the kind permission of the herbarium curators. All vouchers were photographed so that the DNA sequence data could be linked to imaged specimens. Preference for a sample was always given to vouchers annotated by regional or generic experts. Besides, we supplemented missing data with 175 sequences from GenBank and Barcode of Life Data System (with necessary precautions: Funk & al., 2018) of species or fragments which complemented our

database. In total, we sampled all genera and 128 of 140 species (91%) in Buxaceae. Only some rare and local species which are underrepresented in herbaria and living collections, were not sampled in our DNA dataset.

We used standard approaches for DNA extraction and employed commercial DNA extraction kits. DNA was extracted using either a MO BIO PowerPlant DNA Isolation Kit (MO BIO Laboratories, Carlsbad, California, USA) or NUCLEOPIN Plant II Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). Dry plant leaf material (typically, 0.05–0.09 g) was powdered using a sterile mortar and pestle and then processed in accordance with the supplied protocol. We increased the lysis time to 30 minutes and used thermomixer on the slow rotation speed (350 rpm) instead of a water bath. Nanodrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to assess the concentration and purity (the 260/280 nm ratio of absorbance) of DNA samples. In our phylogenetic trees, we decided to integrate data from “barcoding” markers: plastid *rbcL* plus *trnL*-F spacer and nuclear ITS to represent both chloroplast and nuclear genomes. Fortunately, herbarium specimens of Buxaceae typically retain DNA of relatively good quality for many years (Choi & al., 2015). If the particular sample did not yield a sequence of good quality, we tried to use another sample of the same species.

We sequenced the markers mentioned above using primers and protocols in accordance with recommendations of the Barcoding of Life Consortium (Kuzmina & Ivanova, 2011). PCR was carried out as follows: the reaction mixture in a total volume of 20 µL contained 5.2 µL of PCR Master Mix (components from QIAGEN Corporation, Germantown, Maryland, and Thermo Fisher Scientific, Waltham, Massachusetts for Platinum DNA Taq Polymerase), 1 µL of 10 µM forward and reverse primers, 2 µL of DNA solution from the extraction above and 10.8 µL of either MQ purified water (obtained from a Barnstead GenPure Pro system, Thermo Scientific, Langenselbold, Germany), or the TBT-PAR water mix (Samarakoon & al., 2013). Samples were incubated in a thermal cycler: 94° for 5 min, then 35 cycles of 94° for 1 min; 51° (or similar, annealing temperature was varied with a primer) for 1 min, 72° for 2 min, and finally 72° for 10 min. Single-band PCR products were sent for purification and sequencing to Functional Biosciences, Inc. (Madison, Wisconsin) and sequenced there in accordance with standard protocol. Sequences were obtained, assembled, and edited using Sequencher™ 4.5 (Genes Codes Corporation, Ann Arbor, Michigan, USA) and then aligned with AliView (Larsson, 2014) and MUSCLE (Edgar, 2004).

For all procedures and statistic calculations, the R environment (R Core Team, 2019) was used. We used Pipeline (Shipunov, 2020), the R-based DNA sequence analysis pipeline for databasing, sequence analysis, and phylogeny estimation. Pipeline is the combination of UNIX shell scripts and R scripts which allows for (a) species name checks using taxonomy database, (b) cross-validation of sequences, (c) updates from GenBank, (d) completeness analysis and species accumulation control, (e) deselection and replacement of outliers (both on the level of sequences and on the level of trees), (f) sequence alignments using the external tools, (g) flank cleaning, (h) gap coding based on Borchsenius (2009) algorithm which uses simple gap coding *sensu* Simmons and Ochotorena (2000), (i) smart (strict and semi-strict) concatenation (supermatrix production), and (j) a wide variety of phylogenetic outputs, from the k-mer alignment-free to Bayesian and maximal likelihood analyses. In addition, Pipeline is capable of using morphological characters, perform nearest neighbor imputation of missed sequences, and producing super-alignments (Ashkenazy & al., 2018). The Pipeline is available from the primary author's Github: <https://github.com/ashipunov/Pipeline>. Within Pipeline, model testing and phylogenetic trees were made with APE (Paradis & al., 2004), MrBayes (Ronquist & Huelsenbeck, 2003), ips (Heibl, 2008), phangorn (Schliep, 2011) and RAxML (Stamatakis, 2014).

Before the alignment, sequence sets were constructed with the principle that sequences produced for this study had priority, and external data were added only to fill sampling gaps or to replace sequences of unreliable quality. This reduced any possible discrepancies based on incorrect identification and absence of proper vouchers in public databases (Funk & al., 2018). Phylogenetic tree construction within Pipeline used both individual markers and their combinations (supermatrices, concatenated sequences). The preference was given to the concatenation of two sequences that originated from one (our) sample (strict concatenation). On the next (semi-strict) stage, sequences with the same species name that were not derived from the same sample were concatenated.

Using Pipeline, we were able to obtain maximum likelihood (ML), Bayesian (MB), and maximum parsimony (MP) phylogenetic trees. Maximum likelihood analyses were run RAxML (Stamatakis, 2014) with 10,000 bootstrap replicates and R ips package (Heibl, 2008). We used a GTR+G+I model based on model testing with R phangorn package (Schliep, 2011). Bayesian analyses were run through the combination of MrBayes 3.2.6

(Ronquist & Huelsenbeck, 2003), and R shipunov package (Shipunov, 2019). MCMC chains were run for 1,000,000 generations, sampling every 10th generation resulting in 100,000 trees. The first 25% of trees were discarded as burn-in, and the remaining trees were summed to calculate the posterior probabilities. The convergence of Bayesian analyses was controlled using the standard deviation of frequencies across runs, and the potential scale reduction factor, PSRF (Ronquist & Huelsenbeck, 2003). Maximum parsimony analyses were run with the help of R phangorn package (Schliep, 2011) using parsimony ratchet (Nixon, 1999) with 2000 iterations and then 1000 bootstrap replicates. With the aid of the R ape package (Paradis & al., 2004), trees were rooted with *Trochodendron aralioides* and *Tetracentron sinense* as outgroups. To assess the congruence between chloroplast and nuclear data, we used the CADM test (Campbell & al., 2011).

We used two kinds of supermatrices. The first was based on plastome (hereafter, CP) sequences only and included four chloroplast regions: *rbcL*, *trnL-F*, *matK* and *petD*. The second supermatrix (hereafter, OI) included two chloroplast sequences (*rbcL* and *trnL-F*) and also ITS2. Our plastome (CP) dataset was longer (5631 bp including 589, 1303, 2614, and 1125 bp of *rbcL*, *trnL-F*, *matK*, and *petD* parts, respectively) but covered less taxonomic diversity: all genera and 72 species (51%). This matrix, therefore, follows the “more genes” approach (Rokas & Carroll, 2005). The second matrix (OI), was shorter (2581 bp including 689 bp ITS) and was generated mostly from our data that covered all genera and 128 species (91%) of the Buxaceae. To help with *Pachysandroideae* phylogeny estimation, we produced the third matrix (hereafter, “full ITS”), which uses full ITS sequences (generally, ITS1, 5.8S, and ITS2). This matrix covered five genera and 29 species of Buxaceae and was 1371 bp in length.

Datasets, scripts, and phylogenetic trees used in the preparation of this publication are available from the first author’s Open Repository here: <http://ashipunov.info/shipunov/open/buxineae.zip>. We encourage readers to reproduce our results and develop our methods further. All sequences were deposited into the GenBank (Support Table 3).

In the paper, we followed the “appropriate citation of taxonomy” (ACT) principle (Seifert & al., 2008) and cited names of the most supra-species groups (Reveal, 2011, 2012) plus those species which are separately discussed.

## Results

In total, we obtained 359 sequences from 118 species (Support Table 3) and sequenced 50 species of Buxaceae for the first time. Of the resulting matrices that were analyzed, the average percentage of the data produced for this study (*versus* data which came from public databases) was near 74%.

CADM test for the congruence between *rbcL* + *trnL* and ITS parts of OI supermatrix returned the average Kendall concordance value (W=0.53576877), the null hypothesis of incongruence was rejected with p-value 0.01598402 (Chi-squared=11341.15325383). In contrast to Rossello et al. (2007), we did not find issues with multiple copies of ITS present in samples.

In essence, MB, ML, and MP analyses resulted in very similar trees, and overall phylogeny is almost identical in MB and ML (Figs. 2–3). Below, we describe our results based on MB analyses of CP and OI supermatrices (Figs. 4–5), and ML analysis of “full ITS” matrix (Fig. 6). All trees are accessible from the open repository.

### *Didymelis* and *Haptanthus*

*Didymelis* and *Haptanthus* were consistently recovered as the two first branches (Figs. 2–5). The Buxaceae as a whole, as well as a node next to *Didymelis* + *Haptanthus* grade, was supported well (BPP > 96%) on CP and OI trees. A morphologically distinct (Fig. 7) sample of *Didymelis* from Toamasina (Madagascar) recovered as a first branch in the *Didymelis* clade (Fig. 4–5).

### *Buxus*

*Buxus* s.l. is recovered as monophyletic with high support (Figs. 2–5). We sequenced 38 species of *Buxus* (35%) for the first time. Within *Buxus*, three clades were recovered with as monophyletic (Figs. 4–5): an African clade with BPP 84–91% across analyses, an American clade typically highly (BPP 100%) supported, and a Eurasian clade with BPP 86–100%.

The Eurasian clade of *Buxus* is not resolved well. Several South Asian species formed a basal subclade with low support (Fig. 5). This clade includes *Buxus sirindhorniana* W.K. Soh et al. from Thailand, *B. rolfei* S.Vidal and

an unidentified sample from the Philippines, and the sample of unknown origin labeled in IBSC as “*Buxus celebica* Hats.” Two other stable Asian subclades recovered (Fig. 5) in OI analyses, one consisted of *B. myrica* H.Lév., *B. hainanensis* Merr., *B. latistyla* Gagnep., and *B. austroyunnanensis* Hatus.; and another which was comprised of *B. stenophylla* Hance and *B. megistophylla* H.Lév.

Within the African *Buxus* clade (Figs. 4–5), species which were sometimes described under *Notobuxus* (like *Buxus cordata* (Radcl.-Sm.) Friis) were present together with species from *Buxus* s.str. (like *B. hildebrandtii* Baill. and *B. madagascarica* Baill.). Support of smaller clades varied but BPP typically was above 50%.

The American clade was the most speciose in our datasets. The first branches form a grade, which includes *B. bartlettii* Standl., *B. pubescens* Greenm., *B. moctezumae* Eg.Köhler, R.Fernald & Zamudio and *B. mexicana* Brandegee. *Buxus jaucoensis* Eg.Köhler does not hold a stable position on our trees; it is frequently sister to most or all subclades. The next branches were mostly Cuban species (for example, *Buxus koehleri* P.A.González & Borsch, *B. flavidamea* (Britton) Mathou, *B. obovata* Urb., *B. vaccinioides* (Britton) Urb.). The last big subclade includes many non-Cuban species, for example, *Buxus portoricensis* Alain and *B. citrifolia* (Figs. 4–5). Some Cuban species (for example, *Buxus* “*rotundifolia*”, see below about this name) also branch here.

#### *Pachysandroideae* Record et Garratt

The results of all phylogenetic analyses of the matrices recovered a monophyletic *Pachysandroideae* (Figs. 2–6). *Sarcococca* was not monophyletic on CP and “full ITS” trees, and the New World *S. confertifolia* was either robustly placed as sister to the other genera of the subfamily (“full ITS”), as sister to *Styloceras* + *Pachysandra* (CP) with high support, or (OI) as sister to the remaining species of *Sarcococca* (with low support).

Seven species of *Sarcococca* were sequenced for the first time (Fig. 5). There was high support (OI) for *S. confertifolia* Sealy, *S. longipetiolata* M. Cheng, *S. orientalis* C.Y. Wu, and *S. philippinensis* Stapf ex Sealy group. This clade was the first branch in *Sarcococca* s.str., and the remainder of the *Sarcococca* species formed another stable clade. This last clade includes the recently described Vietnamese *S. bleddynii* J.M.H.Shaw & N. van Du (Shaw, 2011), *S. euphlebia* Merrill from Hainan, unidentified *Sarcococca* sp. from Myanmar as a sister group to *S. zeylanica* Baill., and seven other species.

*Pachysandra* was not recovered as monophyletic in all analyses. Whereas a monophyletic *Pachysandra* presents in CP analyses with low support (Fig. 4), it was not monophyletic on OI and “full ITS” trees. On OI trees, *Pachysandra terminalis* was recovered as sister to *Styloceras* with high support (Fig. 5), and on “full ITS” trees, it was placed between *Sarcococca connattii* and the remainder of the *Pachysandroideae*, albeit with low support (Fig. 6).

Four (out of six) species of *Styloceras* were sequenced for the first time. On OI trees, *Styloceras* is monophyletic and well-supported (Fig. 5). *Styloceras laurifolium* (Willd.) Kunth is sister to five other species of the genus with the reliable support; two other clades consisted of *S. kunthianum* A. Juss., *S. penninervium* A.H. Gentry & G.A. Aymard and *S. connatum* Torrez & P. Jørg. (BPP 94%) and *S. columnare* Müll.Arg. + *S. brokawii* .H.Gentry & R.B.Foster (BPP 100%). An unidentified sample of *Styloceras* from Oxapampa (Peru) is morphologically similar to *S. penninervium* and was placed sister to it on OI trees (Fig. 5).

## Discussion

Our dataset provides the most broadly sampled phylogenetic analyses of Buxaceae to date. In some groups, the amount of molecular information is doubled, and even tripled (*Styloceras*). Our molecular phylogenetic results support the elevation of *Sarcococca connattii* into a new genus, *Sealya* (described below).

### ***Didymeles* and *Haptanthus***

Buxaceae *sensu lato* is robustly supported in all our analyses (Figs. 2–5). *Didymeles* and *Haptanthus* form two earliest lineages sister to the remainder of Buxaceae. The analyses resulted in an equivocal placement of *Haptanthus* and *Didymeles*, which is in agreement with the earlier analyses (Shipunov & Shipunova, 2011). This instability *versus* the stability of Buxaceae *sensu lato* provides an indirect support of the integrity of the whole group. As both our results and the morphology of these two distinct genera support a close relationship to the core Buxaceae (Oskolski & al., 2015; Takahashi et al., 2017), we include them in the Buxaceae *sensu lato* as distant, early diverging lineages. This inclusion necessitates the description of the two new subfamilies in Buxaceae (see below).

## *Buxus*

In *Buxus* s.l. three distinctive clades were recovered that correspond perfectly to their geographic distributions.

The Eurasian species were sister to the remaining *Buxus* comprised of a New World and sub-Saharan African clade sister to one another. This topology is similar to that of von Balthazar & al. (2000) and Gutiérrez (2014), and all newly sequenced data fit well with these three geographical clades.

Classification of *Buxus* as a single genus vs. multiple genera is still contentious. The recognition of *Notobuxus* separate from *Buxus* is frequently but not totally accepted (*cf.* Friis, 1989; von Balthazar et al., 2000; Köhler, 2007, 2009). At the same time, the monophyletic *Tricera* has often been included in *Buxus* despite being molecularly closer to *Notobuxus* (von Balthazar et al., 2000). A reasonable approach based on the data presented here that does not require extensive nomenclature changes, is to recognize an inclusive *Buxus* with three well-supported monophyletic clades; we treat these Eurasian, African and American clades at a subgeneric level.

The Eurasian *Buxus* are highly diverse in southeastern Asia, with two or more species extending west to Europe (Köhler & Brückner, 1989; van Laere et al., 2011). The recently described *Buxus sirindhorniana* from Thailand (Soh & al., 2014; Soh & Parnell, 2018) and *B. rolfei* from the Philippines are members of the earliest lineage to diverge in the Eurasian *Buxus*. Our topologies (Fig. 4–5) do not recover the proposed infrageneric divisions of Mathou (1940) and Hatusima (1942). Of Hatusima's (1942) informal groups, none are recovered as monophyletic though some of the species that form parts of his groups (namely, “Group I”) do form well-supported clades, e.g., *B. myrica*, *B. hainanensis*, *B. latistyla*, and *B. austroyunnanensis*. This last grouping also corresponds with the more recent review of Tianlu & Brückner (2008).

*Buxus* from Africa and Madagascar have variously been split into distinct genera separate from *Buxus*, or included within a broad *Buxus* (Van Tieghem, 1897; Friis, 1989; Schatz & Lowry II, 2002). Friis (1989) recognized three sections in African *Buxus*: sect. *Tricera* (Swartz ex Schreb.) Baill. including only *B. hildebrandtii*, sect. *Notobuxus* (Oliv.) Friis and sect. *Buxella* (Tiegh.) Hutch. These divisions are also supported by palynology (Köhler & Brückner, 1982; Köhler & Brückner, 1989; Köhler, 1994). Our molecular CP and OI analyses supported the recognition of sect. *Buxella*, excluded *B. hildebrandtii* from New World *Tricera* but did

not fully resolve relationships between *B. hildebrandtii* and “sect. *Notobuxus*” species. We treat the African clade as subg. *Notobuxus*, a single monophyletic group; subclades recovered here might support further sectional divisions.

American *Buxus* clade forms a well-supported monophyletic taxon endemic to the Neotropics with a single migration and rapid diversification in the Caribbean-Cuban *Buxus* species (Köhler, 2014; Gutiérrez, 2014). Our CP trees were in line with Gutiérrez (2014). OI trees were different but overall support was much lower, and we cannot fully exclude the effect of putative ITS paralogues (Rosello et al., 2007; Gutiérrez, 2014). In all, we believe that barcoding markers are not powerful enough in cases of rapid diversification, and this group still awaits detailed research. However, but we were able to place there some newly sequenced species (Fig. 5), for example, *B. lancifolia* and *B. vaccinioides*.

#### *Pachysandroideae*

The *Pachysandroideae* is united by its crotonoid pollen (Gray & Sohma, 1964; Köhler, 2007; see also Fig. 8), but the genera are otherwise morphologically distinctive. Our results of the *Pachysandroideae* present a topology (Figs. 2–6) where the long-questionably placed *Sarcococca connattii* (Fig. 8) was consistently recovered separate from all other species of *Sarcococca*. In our analyses, it branches distantly from the rest of *Sarcococca* (OI, and especially “full ITS”: Figs. 5–6) or as a lineage sister to the *Sarcococca* (CP: Fig. 4). We used molecular data as an additional evidence and herein recognized a novel monotypic genus, *Sealya* (see below).

*Sealya* was nearly concurrently described in both *Buxus* and *Sarcococca* (Standley, 1936; Johnston, 1938), and its inclusion within *Sarcococca* was based on vegetative similarities (alternate, subcoriaceous leaves) and similar fruit types. However, it differs from *Sarcococca* in its inflorescence structure, which is similar to that of *Buxus* in having a terminal female flower; furthermore, its terminal female flowers possess the well-developed tepals and bracteoles (Sealy, 1986; Köhler, 2007). Its fruits are indehiscent like in *Sarcococca*, but have dry mesocarps and are white in contrast to the *Sarcococca* s.str. blackish-blue or reddish fruits (Sealy, 1986). *Sealya* shares the crotonoid pollen exine (Fig. 8) with all other members of *Pachysandroideae* (Köhler, 2007).

With *Sealya* excluded, *Sarcococca* is monophyletic in all our analyses. Even with our still limited sampling, it appears that the diversity in *Sarcococca* is higher than current taxonomic treatments recognize (Sealy, 1986; Min & Brückner, 2000). For example, our analyses recovered *S. euphlebia* and *S. balansae* separately and distantly placed, but Sealy (1986) placed *S. euphlebia* as a synonym of *S. balansae*. In contrast, Min and Brückner (2000) placed both as synonyms of *S. vagans* (which we did not sample). Inclusion of *S. vagans* might result in one of the preceding species being supported as a synonym, but not both. Additional systematic work is needed with a comprehensive sampling of widespread species to resolve taxonomy in *Sarcococca*.

Despite the low species number and relatively high level of systematic investigation (Robbins, 1968; Boufford & Xiang, 1992; von Balthazar et al. 2000; Jiao & Li, 2009), *Pachysandra* also presents a potential for further studies. Our OI and “full ITS” analyses (Figs. 5–6) point on the possible separation of the *Pachysandra terminalis* from two other species of the genus. Morphologically, it differs from two other species in its terminal inflorescences, two-carpellate gynoecium, the smaller stigmatic region encompassing on the distal third of the style, the male flowers subtended by a coriaceous bract and two bracteoles, elongate multi-bracted pedicels of the pistillate flowers, white baccate fruits, and adaxially raised leaf veins (Robbins, 1968). Future molecular research should sample even more extensively and include *P. terminalis* from both areas of its distribution (Japan and China) as well as the broadly distributed and morphologically diverse *P. axillaris*.

Our coverage of *Styloceras* is comprehensive and represented here by seven samples and six species. *Styloceras laurifolium* holds the most basal position while the rest of the genus form two clades with the reliable support: *S. penninervium* + *S. kunthianum* + *S. connatum*, and *S. brokawii* + *S. columnare*. This topology does not correspond well with existing review (Torrez & Jorgensen, 2010) which places *S. kunthianum*, *S. columnare* and *S. laurifolium* together and separates *S. penninervium* from the rest of the genus. Given the scarcity of information and collections, and difficulties in DNA amplification in *Styloceras*, we believe that this is only a first step towards understanding the diversity of this rare and unusual Neotropical group.

## Taxonomic Treatment

***Didymelis toamasinae* Floden et Shipunov, sp.nov.**

Differs from *Didymelis perrieri* Leandri by smaller leaves ( $30\text{--}50 \times 15\text{--}30$  mm vs.  $50\text{--}100 \times 30\text{--}37$  mm), intensively branching shoots, thinner terminal branches (1.5–2 vs. 2–4 mm diam.), and smaller (12–15 vs. 15–20 mm) mature fruits.

TYPE:—Toamasina. Fivondronana: Maroantsetra. Commume: Antirasbesahatany. Fokontany: Anjiahely. Beanivona forest. Collected with Pascal, Ramaroson, Saside and David.  $15^{\circ}23'56"S$   $045^{\circ}26'32"E$ . 746 m. Tree 8 m tall, DBH 20 cm, fruits nearly mature. 17 December 2002. *P. Antilahimena & al. 1529* (holotype: MO!)

Etymology:—Named after the region of collection, Toamasina, northeastern Madagascar.

Distribution:—MADAGASCAR, northeast, Atsinanana region. (MO *P. Antilahimena 2497a!*, MO *P. Antilahimena, Pascal & Ramaroson 1561!*)

Description:—Small trees 4–8 m, young stems 1–2 mm diam., internodes 2–4 cm. Leaves with petioles 1.2–1.5 cm, laminas  $3\text{--}5 \times 1.5\text{--}3$  cm, elliptic, apex acuminate, base cuneate, with visible primary and secondary venation. Flowers not seen. Fruits 1.2–1.5 cm, ellipsoid, stylar remnants about 2 mm.

***Buxus cyclophylla* Floden & Shipunov, nom.nov.**

As the Cuban *Buxus rotundifolia* (Britton) Mathou is the latter homonym of *Buxus rotundifolia* K.Koch, we propose a new name for the former:

*Buxus cyclophylla* Floden & Shipunov, nom.nov. pro *Buxus rotundifolia* (Britton) Mathou, Rech. Fam. Buxac. 229 (1939). nom.illeg., non *Buxus rotundifolia* Hort. ex K.Koch, Dendrologie 2(2): 479 (1873).

≡ *Tricera rotundifolia* Britton, Bull. Torrey Bot. Club 42: 500 (1915).

Type: — NY Shafer 114! Between Camp La Barga and Camp San Benito, about 1,000 m alt., northern Oriente.

***Sealya* Floden et Shipunov gen. nov.**

Differs from *Sarcococca* by its inflorescence terminated by a female flower (*vs.* male), male and female flowers with well-developed tepals (*vs.* weakly differentiated as perianth organs), bracteoles prominent, and by its white fruit (*vs.* purple to red) with dry mesocarp (*vs.* fleshy mesocarp), while it also differs from *Buxus* by having alternate leaves, bicarpellate ovary (*vs.* tri), and fleshy, drupe-like fruit (*vs.* dry and dehiscent).

Generic type:

*Sealya conzattii* (Standl). Floden et Shipunov **comb. nov.** TYPE:—Mexico, C. Conzatti 2508 (holotype: F digital image!; isotypes: EAP87195 digital image!, G00359411 digital image!, US digital image!).

Basionym:—*Buxus conzattii* Standley, Publications of the Field Museum of Botany, 11: 163 (1936).

SYNONYMS:—*Sarcococca conzattii* (Standley) I. M. Johnston, Journal of the Arnold Arboretum, 20: 240 (1939); *S. guatemalensis* I. M. Johnston, Journal of the Arnold Arboretum, 19: 121 (1938). — Type: Guatemala, A.F. Skutch 288 (holotype: A00048976 digital image!; isotype: K000573596 digital image!, MICH digital image!, TX/LL!, US digital image!)

Etymology:—Named in honor of J. Robert Sealy who stated in his 1986 revision of *Sarcococca* that the generic placement of *S. conzattii* in *Sarcococca* was incorrect and its placement in *Buxus* would be “equally anomalous.”

Distribution:—MEXICO. Chiapas, Oaxaca. GUATEMALA, occurring at mid-to higher elevations.

***Buxus* subgenus *Notobuxus* (Oliv.) Floden et Shipunov, subg.nov.**

Basionym:—*Notobuxus* Oliv. in Hook, Ic. Pl. 14:78, t 1400. 1882. Type:—*B. natalensis* (Oliv.) Hutch., Africa, Natal, Inanda, Wood 1357 (lectotype (designated here), K, isolectotypes, BOL136821, BOL136822, NH0001717-1, NH0001717-2).

Subgenus *Notobuxus* has been repeatedly shown in molecular studies to be a monophyletic group of African and Malagasy species (Gutiérrez, 2014; von Balthazar & al., 2000). It is characterized by frequently sessile anthers, and by

the frequent presence of multiple (6–10) stamens and flat disk-shaped pistillode (Friis, 1989; von Balthazar & Endress, 2002a; Köhler, 2007). *Notobuxus* Oliv. was based on two separate collections that represent syntypes because no type was designated (Article 9.3: Turland & al., 2018). From the original material (Article 9.4) cited by Oliver (1882) there was a collection with unopened flowers (*T. Cooper* 3465, K) and a collection by *Wood* 1357 with opened flowers. The accompanying illustration (Oliver, 1882: Plate 1400) was drawn from the latter, thus we select *Wood* 1357 to serve as the lectotype.

***Buxus* subgenus *Tricera* (Swartz ex Schreb.) Floden et Shipunov, subg.nov.**

Basionym: —*Tricera* Swartz ex Schreb., Gen. 630. 1791. Type:—*B. laevigata* (Sw.) Spreng. Jamaica, O.P. Swartz (holotype S, isotypes G, LD).

Subgenus *Tricera* is wholly New World and differs from the subgenus *Buxus* and subgenus *Notobuxus* by the absence of cortical vascular bundles in the angle of the branchlets (Köhler, 2007). The results of the molecular phylogeny support subg. *Tricera* as a robust monophyletic group endemic to the New World.

***Didymeloideae* Floden & Shipunov, subfam. nov.**

*Didymeloideae* Floden & Shipunov, subfam. nov. – Type: *Didymeles* Thouars

Distribution. – Madagascar.

Genera (1). – *Didymeles* Thouars

***Haptanthoideae* Floden & Shipunov, subfam. nov.**

*Haptanthoideae* Floden & Shipunov, subfam. nov. – Type: *Haptanthus* A. Goldberg & C. Nelson

Distribution. – Central America, Honduras, Atlantida province.

Genera (1). – *Haptanthus* A. Goldberg & C. Nelson

## ***Kichijiso* Floden et Shipunov gen. nov.**

Differs from *Pachysandra* by its terminal inflorescences, gynoecium with two-carpels, a stigmatic region encompassing only the distal third of the style, male flowers subtended by a coriaceous bract and two bracteoles, the elongate multi-bracted pedicels of the pistillate flowers, its white baccate fruits, and its adaxially raised leaf veins.

**Etymology:** The generic name, *Kichijiso*, is one of the Japanese common names of this plant (Batchelor & Miyabe, 1893: “Kichjisô, the fruit of which are eaten raw”). This selection of the generic name follows other Latinized generic names such as *Aucuba*, *Kirengeshoma*, *Nandina*, and *Sasa* (Stearn, 2004).

**Distribution:** Japan and eastern China (Min Tianlu & Brückner, 2008)

**Generic type:**

***Kichijiso terminalis* (Sieb. et Zucc.) Floden et Shipunov comb. nov.** TYPE:—(lectotype selected by Robbins, 1960) Japonia, 1842, Siebold, P.F. von, s.n. (M-0120840).

**Basionym:**—*Pachysandra terminalis* Siebold & Zuccarini, Abh. Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. 4(2): 142. 1845.

## **Conclusion**

With the broad taxonomic sampling, we provide a comprehensive approach to improve the classification scheme of the Buxaceae. We hope that our results might serve as a framework for future studies in the group, which will eventually reach the highest phylogenetic accuracy. The use of short barcoding molecular markers is likely the best choice for our mostly herbarium-oriented approach. However, at the same time, we argue that more in-depth research that incorporates data on genomics, morphology, anatomy, chemistry, distribution, and fossil history is required to provide overall integrative and synthetic revisions of the genera of the Buxaceae.

## **Key to the Buxaceae *sensu lato***

- 1 Plants dioecious; flowers apetalous; male with one stamen pair; female flowers paired, carpels single and uniovulate, seeds exalbuminous ... ***Didymeloidae*** (subfam. *Didymeloideae*)
  - Plants monoecious; flowers with weakly differentiated perianth parts; male flowers with decussate tepals, 2, 4, or 6–10 stamens; female with spiraled tepals, carpels bi- to pluri-ovulate, seeds albuminous ... 2
- 2 Flowers apparently naked; male flowers with two stamens fused into one staminate organ; female flowers 3-carpellate, carpels pluri-ovulate (8–15), placentation parietal ... ***Hapanthus*** (subfam. *Hapanthoideae*)
  - Flowers with perianth; male flowers with free stamens, female flowers 2–3-carpellate, carpels bi-ovulate, placentation axile ... 3
- 3 Tepals absent in male flowers, stamens numerous; rudiment of ovary wanting ... ***Styloceras*** (subfam. *Pachysandroideae*)
  - Tepals present; stamens usually 4, rarely 6–10 ... 4

4 Leaves decussate; female flowers terminal in racemes or clusters; fruit a 3-valved capsule ... 5 (*Buxus*, subfam. *Buxoideae*)

– Leaves alternate; female flowers at base of racemes or spikes; fruit more or less drupaceous ... 7 (the rest of subfam. *Pachysandroideae*)

5 Cortical vascular bundles wanting (American) ... *Buxus* subg. *Tricera*

– Cortical vascular bundles in each angle of the branches ... 6

6 Cortical vascular bundles with fibre strands; male flowers with 4 stamens, anthers long exserted; pistillode present ... *Buxus* subg. *Buxus*

– Cortical vascular bundles without fibre strands; male flowers with (4) 6–10 stamens; anthers usually sessile; pistillode as a flat disk, or absent ... *Buxus* subg. *Notobuxus*

7 Woody shrubs or small trees; leaves entire; fruit more or less drupaceous ... 8

– Perennial herbs with procumbent stems; leaves serrate to dentate or deeply toothed; flowers borne at the base of the stem or terminally; fruit an indehiscent capsule or subdrupaceous ... 9

8 Female flowers lateral on inflorescences; gynoecium 2- or 3-carpellate; fruit drupaceous, reddish, purple, or blackish ... *Sarcococca*

– Female flowers terminal on inflorescences; gynoecium 2-carpellate; fruit with dry mesocarp, white ... *Sealya*

9 Inflorescences at base of stem or proximal axils; gynoecium 3-carpellate; fruits reddish-brown, indehiscent capsule ... *Pachysandra* (*P. axillaris* and *P. procumbens*)

– Inflorescences terminal; gynoecium 2-carpellate; fruits white, subdrupaceous ... *Pachysandra terminalis* (*Kichijiso terminalis*)

## Author Contributions

AS provided the design of the research; AF and AS performed data analysis, interpretation and writing of the manuscript; all co-authors participate in data collection.

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## **Figure legends**

**Figure 1.** The map of the world which shows the geographic distribution of the group.

**Figure 2.** Comparison of the topologies resulted from the phylogenetic analyses of CP (plastid markers) supermatrix: Bayesian (left) and RAxML (right). Each triangle is the result of concatenation applied to the branches of the corresponding phylogenetic trees.

**Figure 3.** Comparison of the topologies resulted from the phylogenetic analyses of OI (*trnL*, *rbcL* and ITS2) supermatrix: Bayesian (left) and RAxML (right). Each triangle is the result of concatenation applied to the branches of the corresponding phylogenetic trees.

**Figure 4.** The Bayesian phylogenetic tree obtained from CP (plastid markers) supermatrix, node labels denote the BPP (in %). See the text for the explanation of clade names.

**Figure 5.** The Bayesian phylogenetic tree obtained from OI (*trnL*, *rbcL* and ITS2) supermatrix, node labels denote the BPP (in %), stars\* designate species sequenced for the first time. See the text for the explanation of clade names.

**Figure 6.** The maximum likelihood (RAxML) tree obtained from the “full ITS” matrix, node labels denote the bootstrap support, stars\* designate species sequenced for the first time.

**Figure 7.** *Didymelis toamasinae*, the holotype (MO).

**Figure 8.** *Sealya conzattii*, left to right: branch, inflorescence, crotonoid pollen grains (photo credit to Jose Panero, Texas). This plant was also used for DNA extraction.

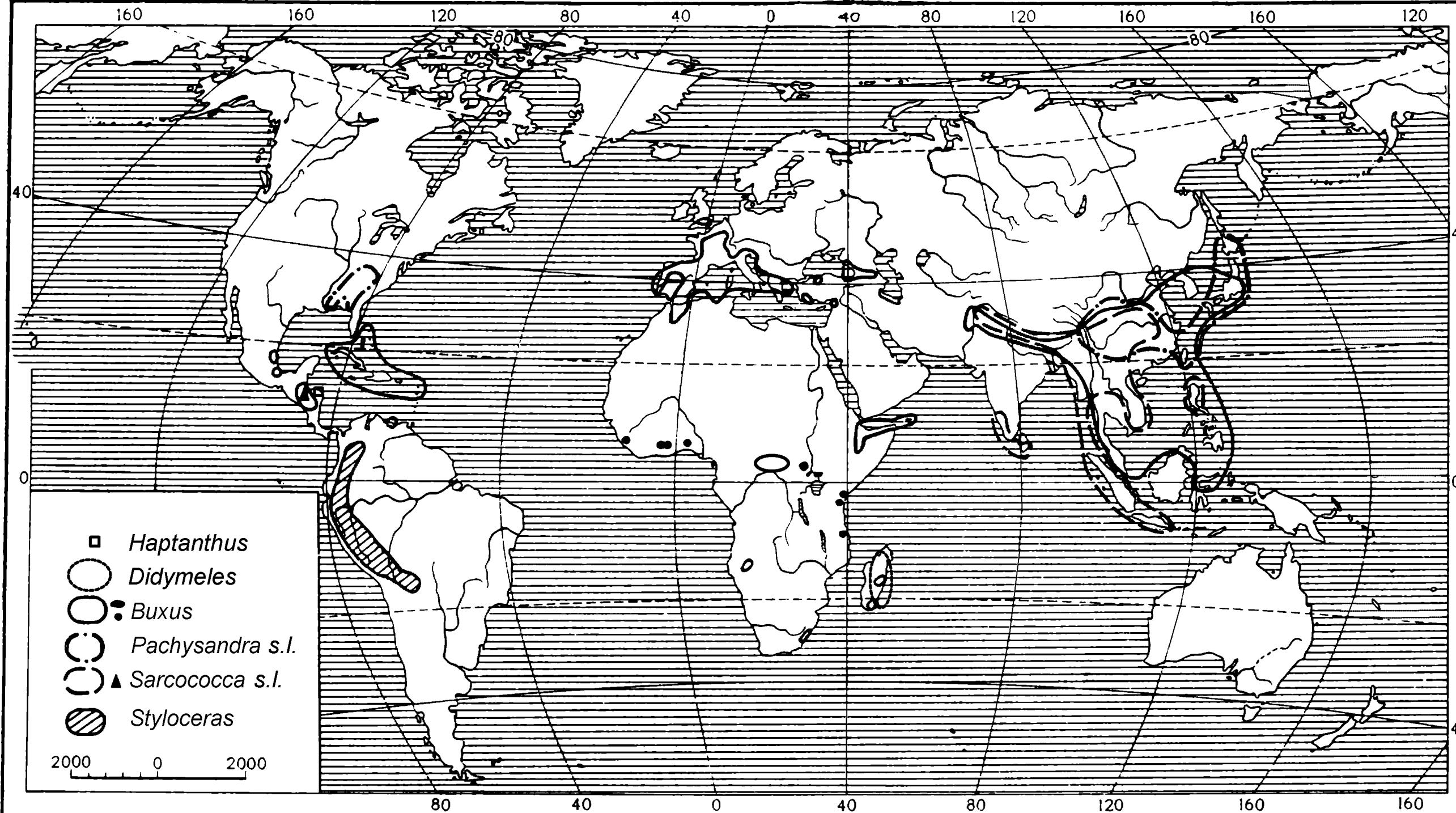
## **Support materials**

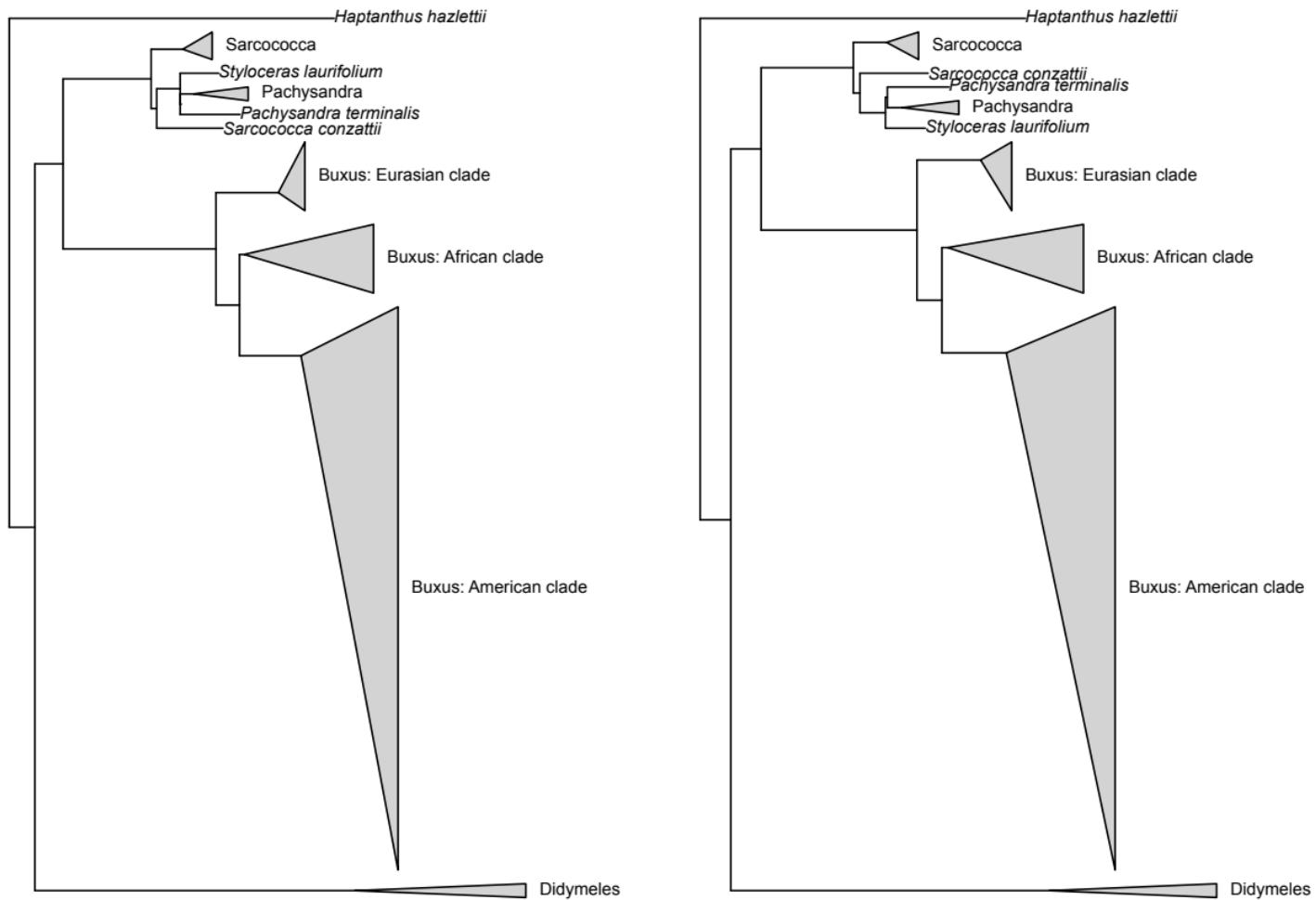
**Support Table 1.** Working classification of Buxaceae.

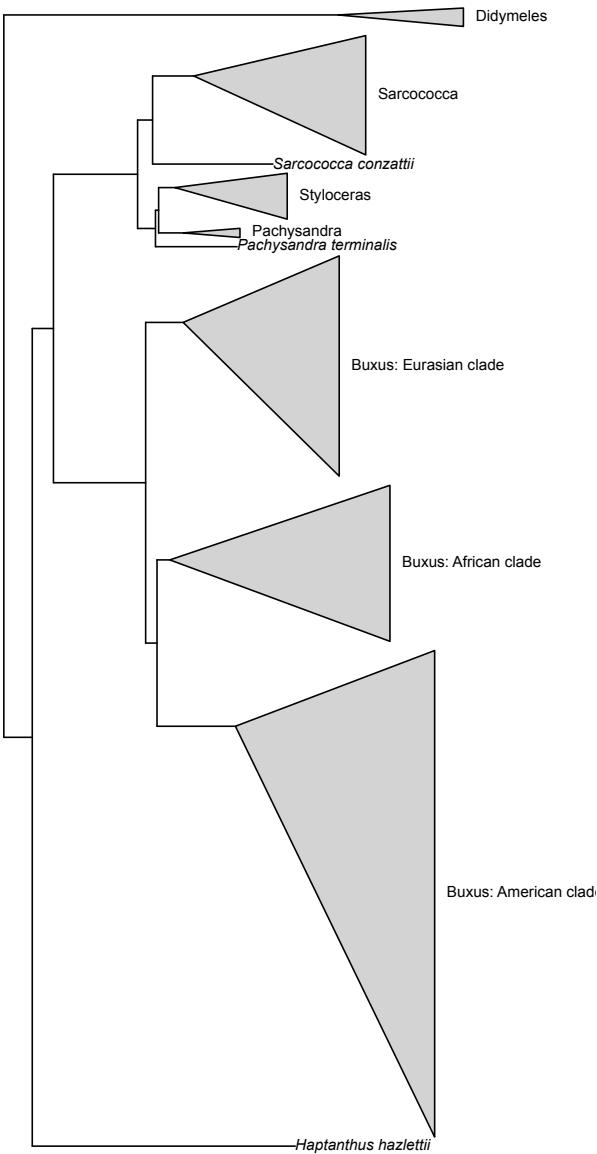
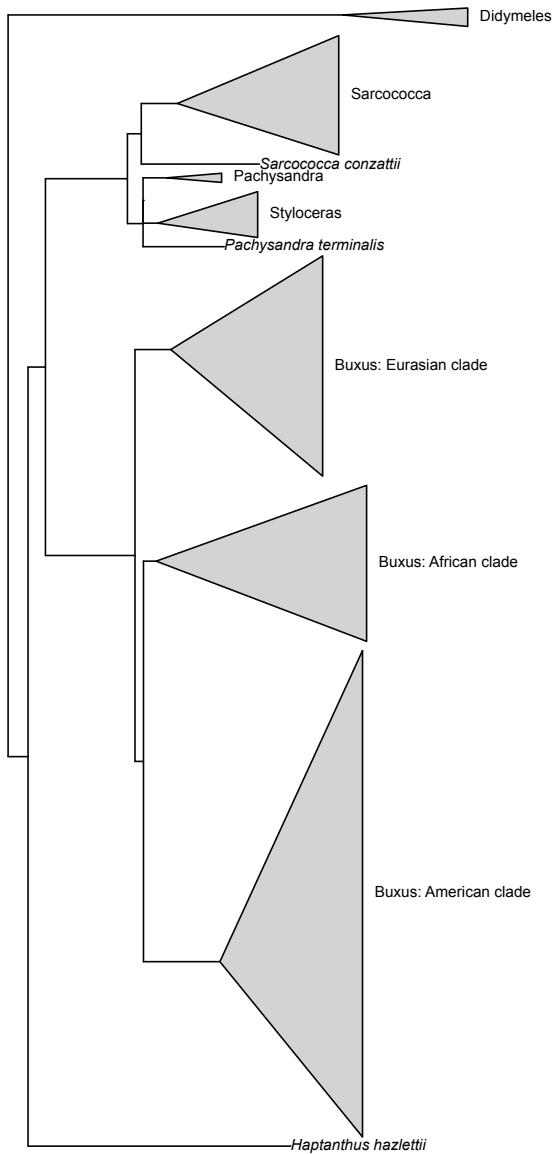
**Support Table 2.** Vouchers of Buxaceae samples.

**Support Table 3.** GenBank accession numbers of Buxaceae samples sequenced for this study.

**Support Table 4.** GenBank accession numbers of Buxaceae sequences of external origin used in this study.







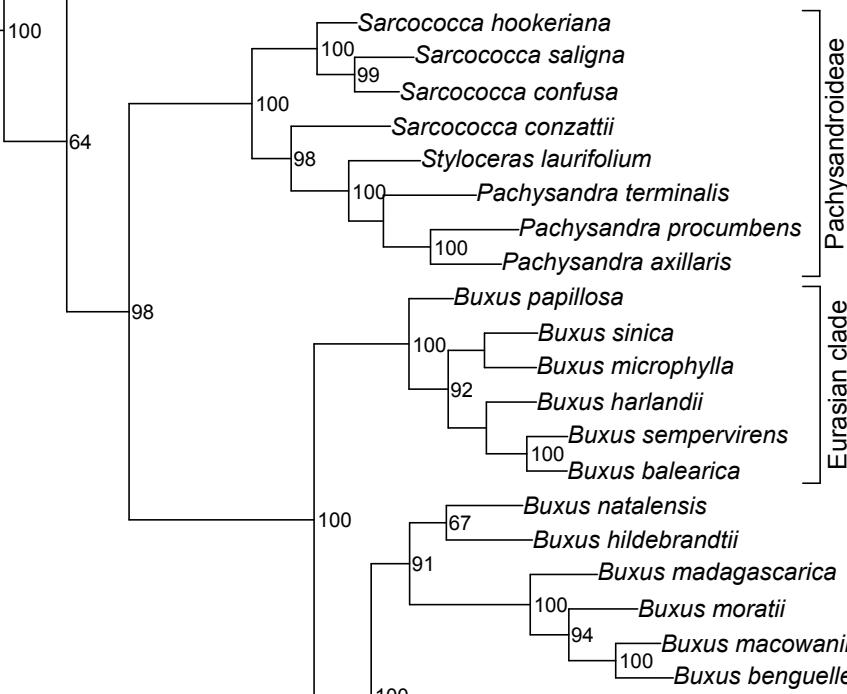
*Tetracentron sinense*  
*Trochodendron aralioides*

Outgroups

*Haptanthus hazlettii*

*Didymelis perrieri*

*Didymelis integrifolia*



0.05

American clade

Tetracentron sinense

Trochodendron aralioides

Outgroups

Didymelis sp.

Toamasina\*

Didymelis perrieri

Didymelis madagascariensis\*

Didymelis integrifolia

Haptanthus hazlettii

Sarcococca confertiflora

Sarcococca philippinensis\*

Sarcococca orientalis

Sarcococca longipetiolata\*

Sarcococca confusa

Sarcococca euphlebia\*

Sarcococca ruscifolia

Sarcococca wallichii

Sarcococca hookeriana

Sarcococca bleddynii\*

Sarcococca balansae\*

Pachysandra terminalis

Styloceras laurifolium

Styloceras kunthianum\*

Styloceras sp. Peru Oxapampa\*

Styloceras penninervium\*

Styloceras connatum\*

Styloceras columnare\*

Styloceras brokawii

Pachysandra procumbens

Pachysandra axillaris

Buxus sp. Philippines\*

Buxus "celebica"\*\*

Buxus sirindhorniana\*

Buxus rolfei\*

Buxus pubiramea\*

Buxus stenophylla\*

Buxus megistophylla\*

Buxus sinica

Buxus microphylla

Buxus liukiuensis

Buxus henryi

Buxus harlandii

Buxus chaoanensis\*

Buxus wallichiana\*

Buxus rugulosa\*

Buxus cephalantha\*

Buxus ichagensis\*

Buxus mollicula\*

Buxus bodinieri\*

Buxus sempervirens

Buxus balearica

Buxus papillosa

Buxus cochinchinensis\*

Buxus myrica\*

Buxus hainanensis\*

Buxus latistylo\*

Buxus austroyunnanensis\*

Buxus itremoensis\*

Buxus madagascariaca

Buxus rabenantandroi\*

Buxus macrocarpa\*

Buxus cipolinica\*

Buxus monticola\*

Buxus capuronii\*

Buxus calcarea\*

Buxus macowanii

Buxus humbertii\*\*

Buxus nyasica\*

Buxus moratii

Buxus benguellensis

Buxus hildebrandtii

Buxus natalensis

Buxus cordata\*

Buxus obtusifolia\*

Buxus acutata

Buxus jaucoensis

Buxus mexicana

Buxus heterophylla\*

Buxus gonoclada

Buxus brevipes

Buxus lancifolia\*

Buxus moctezumae

Buxus pubescens

Buxus bartlettii

Buxus muelleriana

Buxus crassifolia

Buxus koehleri

Buxus retusa

Buxus pilosula

Buxus nipensis

Buxus triptera

Buxus serpentinicola

Buxus cristalensis

Buxus foliosa

Buxus revoluta

Buxus flaviramea\*

Buxus braimbridgeorum

Buxus shaferi

Buxus laevigata\*

Buxus imbricata

Buxus excisa

Buxus leivae

Buxus pseudaneura

Buxus yunquensis

Buxus moana

Buxus obovata\*

Buxus pulchella\*

Buxus glomerata

Buxus wrightii

Buxus vahlii

Buxus bahamensis

Buxus vaccinoides\*

Buxus aneura

Buxus subcolumnaris

Buxus citrifolia

Buxus "rotundifolia"

Buxus rheedioides

Buxus portoricensis

Buxus ekmanii

Buxus acunae

Buxus leonii\*

Buxus purdieana\*

Buxus macrophylla\*

Buxus arborea

Buxus bissei

Buxus sclerophylla

Buxus olivacea

Buxus marginalis

Buxus acuminata

100

68

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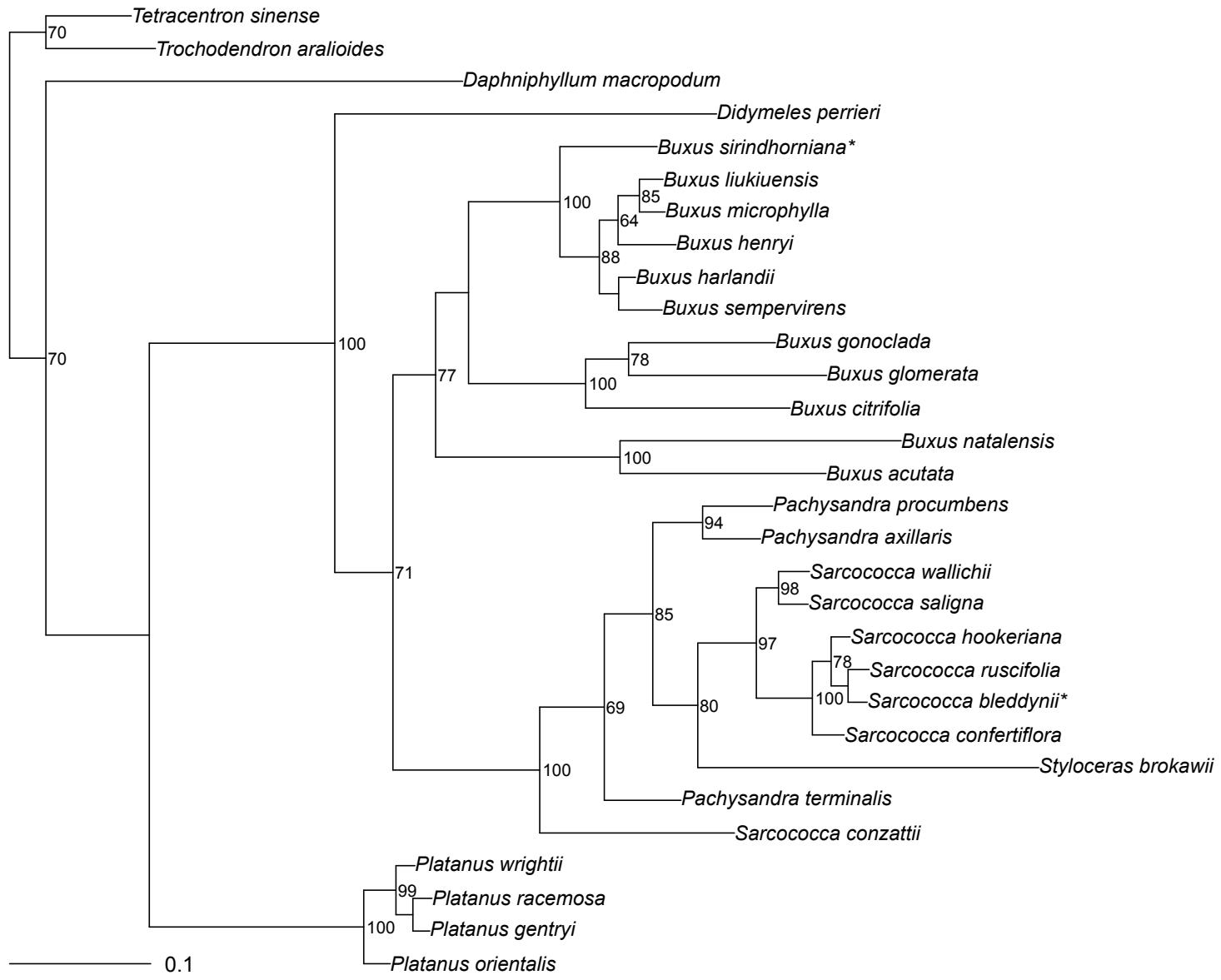
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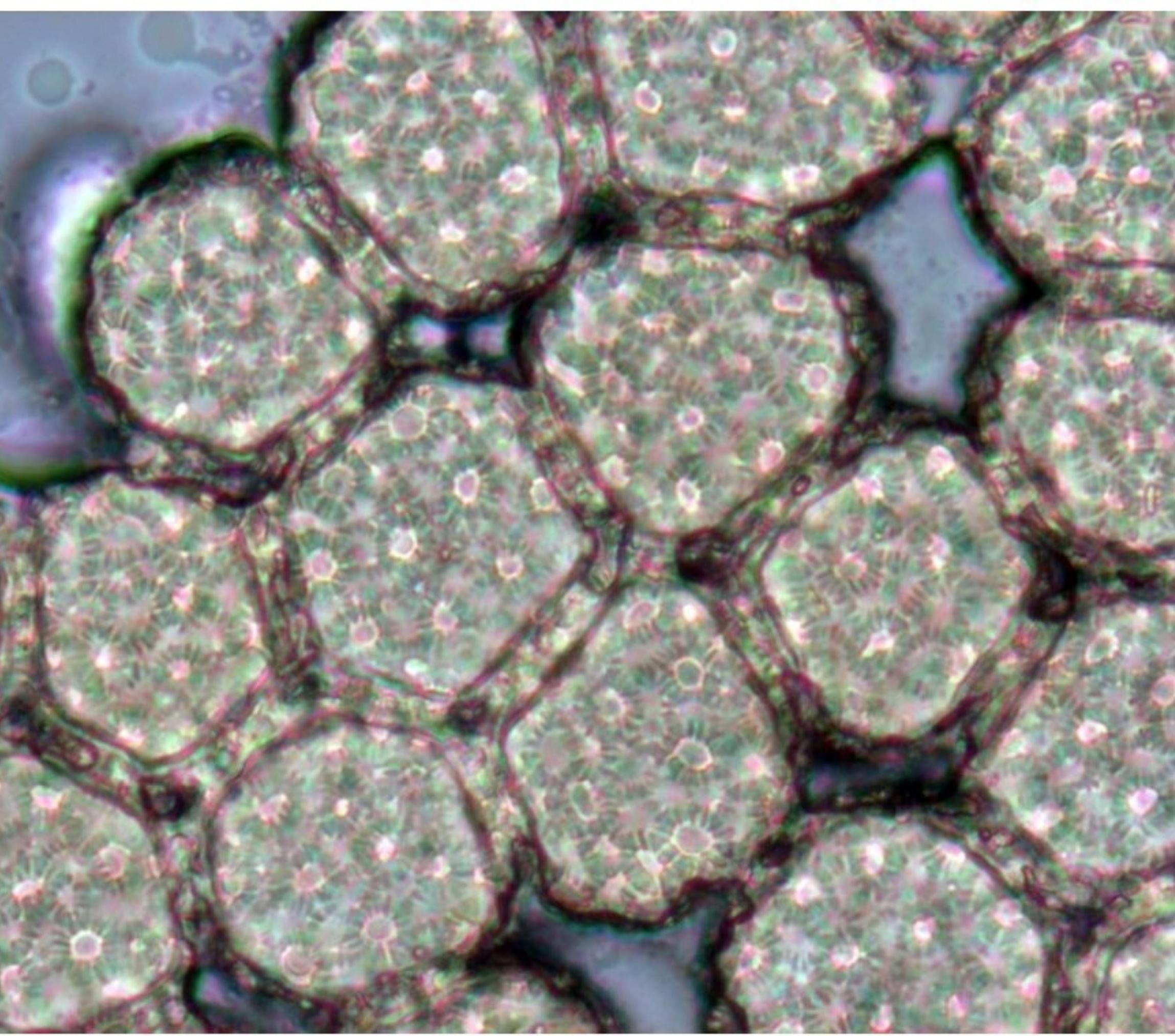
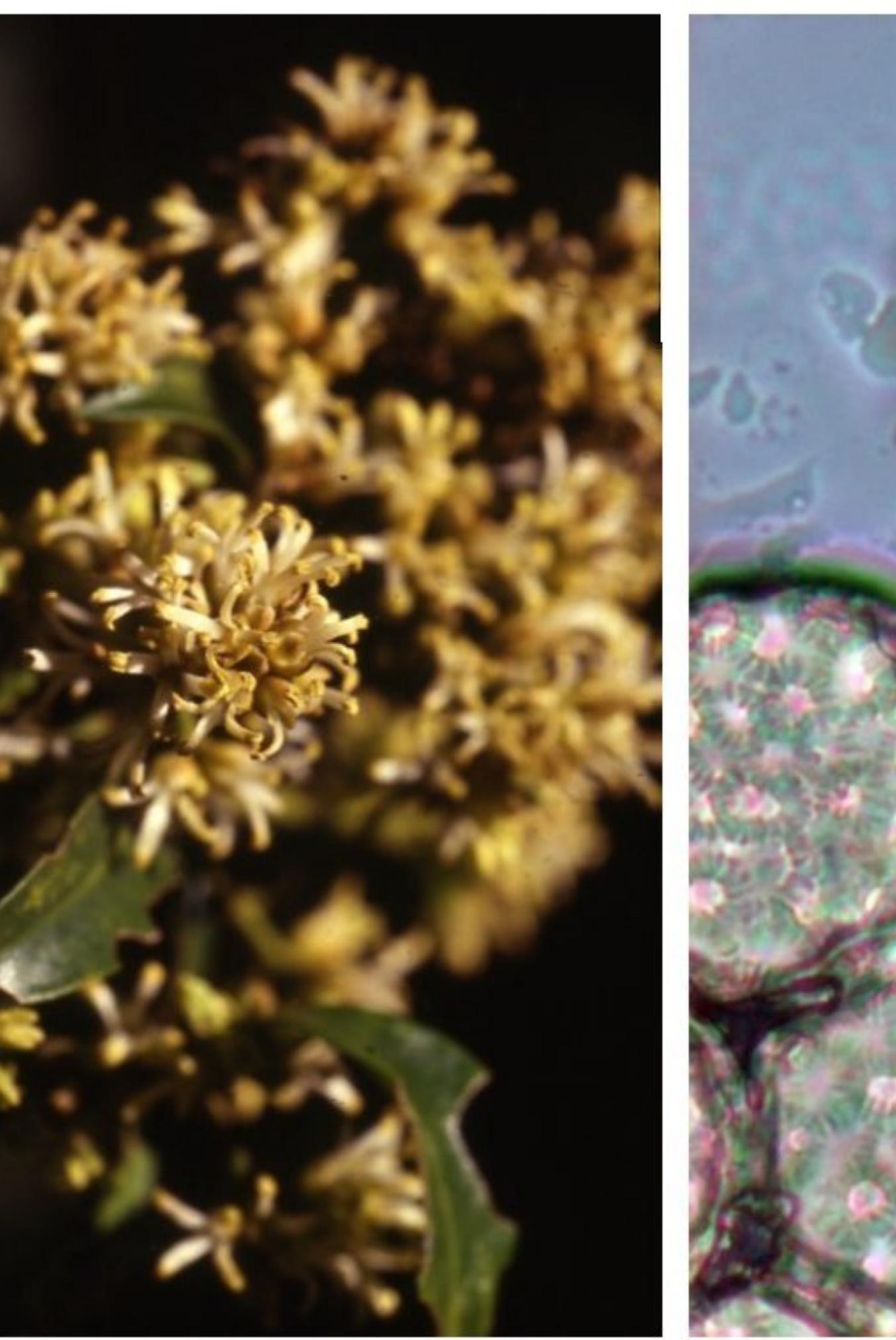
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Buxaceae Dumort.  
Working classification

Alexey Shipunov,  
Aaron Floden

2020-03-24

**Familia 1. Buxaceae** Dumort.

**Subfamilia *Didymeloideae*** Floden & Shipunov, subfam.nov.

Genus 1. DIDYMELES Thouars, stat.m.<sup>1</sup>

- 1(1). *Didymeles integrifolia* J.St.-Hil.
- 2(2). *Didymeles madagascariensis* Willd., stat.m.<sup>2</sup>
- 3(3). *Didymeles perrieri* Leandri
- 4(4). *Didymeles toamasinae* Floden & Shipunov, sp.nov.

**Subfamilia *Haptanthoideae*** Floden & Shipunov,  
subfam.nov.

Genus 2. HAPTANTHUS Goldberg & C.Nelson stat.m.<sup>3</sup>

- 1(5). *Haptanthus hazlettii* Goldberg & C.Nelson

**Subfamilia *Buxoideae*** Beilschm.

Genus 3. BUXUS L.

Subgenus *Buxus*<sup>4</sup>

- 1(6). *Buxus austroyunnanensis* Hatus.
- 2(7). *Buxus balearica* Lam.<sup>5</sup>
- 3(8). *Buxus bodinieri* H.Lév.
- 4(9). *Buxus chaoanensis* H.G.Ye
- 5(10). *Buxus cochinchinensis* Pierre ex Gagnep.<sup>6</sup>

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<sup>1</sup>Sometimes in Didymelaceae Leandri.

<sup>2</sup>*Didymeles excelsa* Baill.

<sup>3</sup>Sometimes in Haptanthaceae C.Nelson.

<sup>4</sup>*Eubuxus* Baill.

<sup>5</sup>*Buxus longifolia* Boiss.

<sup>6</sup>*Buxus holttumiana* Hatus.

- 6(11). *Buxus fortunei* Carruth.  
 7(12). *Buxus hainanensis* Merr.  
 8(13). *Buxus harlandii* Hance<sup>7</sup>  
 9(14). *Buxus hebecarpa* Hatus.  
 10(15). *Buxus henryi* Mayr  
 11(16). *Buxus ichagensis* Hatus.  
 12(17). *Buxus latistyla* Gagnep.  
 13(18). *Buxus linearifolia* M.Cheng  
 14(19). *Buxus liukiuensis* (Makino) Makino  
 15(20). *Buxus loheri* Merr.  
 16(21). *Buxus malayana* Ridl.  
 17(22). *Buxus megistophylla* H.Lév.  
 18(23). *Buxus microphylla* Siebold & Zucc.<sup>8</sup>  
 19(24). *Buxus mollicula* W.W.Sm.  
 20(25). *Buxus myrica* H.Lév.  
 21(26). *Buxus pachyphylla* Merr.  
 22(27). *Buxus papillosa* C.K.Schneid.  
 23(28). *Buxus pubifolia* Merr.  
 24(29). *Buxus pubiramea* Merr. & Chun  
 25(30). *Buxus rivularis* Merr.  
 26(31). *Buxus rolfei* S.Vidal<sup>9</sup>  
 27(32). *Buxus rugulosa* Hatus.  
 28(33). *Buxus rupicola* Ridl.  
 29(34). *Buxus sempervirens* L.<sup>10</sup>  
 30(35). *Buxus sinica* (Rehder & E.H.Wilson) M.Cheng<sup>11</sup>  
 31(36). *Buxus sirindhorniana* W.K.Soh & al.  
 32(37). *Buxus stenophylla* Hance  
 33(38). *Buxus wallichiana* Baill.

**Subgenus *Notobuxus* (Oliv.) Floden & Shipunov, subg.nov.**

- 34(39). *Buxus acutata* Friis<sup>12</sup>  
 35(40). *Buxus benguellensis* Gilg<sup>13</sup>  
 36(41). *Buxus calcarea* G.E.Schatz & Lowry  
 37(42). *Buxus capuronii* G.E.Schatz & Lowry  
 38(43). *Buxus cipolinica* Lowry & G.E.Schatz  
 39(44). *Buxus cordata* (Radcl.-Sm.) Friis<sup>14</sup>

<sup>7</sup>*Buxus cephalantha* H.Lév. & Vaniot

<sup>8</sup>*Buxus riparia* (Makino) Makino

<sup>9</sup>*Buxus philippinensis* Rolfe, nom.nud.

<sup>10</sup>*Buxus colchica* Pojark.; *Buxus hyrcana* Pojark.

<sup>11</sup>*Buxus intermedia* Kaneh.

<sup>12</sup>*Notobuxus acuminata* (Gilg.) Hutch.

<sup>13</sup>*Notobuxus benguellensis* (Gilg) E.Phillips; *Buxus hirta* (Hutch.) Mathou

<sup>14</sup>*Notobuxus cordata* Radcl.-Sm.

- 40(45). *Buxus hildebrandtii* Baill.<sup>15</sup>  
 41(46). *Buxus humbertii* G.E.Schatz & Lowry  
 42(47). *Buxus itremoensis* G.E.Schatz & Lowry  
 43(48). *Buxus lisowskii* Bamps & Malaisse  
 44(49). *Buxus macowanii* Oliv.<sup>16</sup>  
 45(50). *Buxus macrocarpa* Capuron  
 46(51). *Buxus madagascarica* Baill.<sup>17</sup>  
 47(52). *Buxus monticola* G.E.Schatz & Lowry  
 48(53). *Buxus moratii* G.E.Schatz & Lowry  
 49(54). *Buxus natalensis* (Oliv.) Hutch.<sup>18</sup>  
 50(55). *Buxus nyasica* Hutch.<sup>19</sup>  
 51(56). *Buxus obtusifolia* (Mildbr.) Hutch.<sup>20</sup>  
 52(57). *Buxus rabenantoandroi* G.E.Schatz & Lowry<sup>21</sup>

**Subgenus *Tricera*** (Swartz ex Schreb.) Floden & Shipunov, subg.nov.

- 53(58). *Buxus acuminata* (Griseb.) Müll.Arg.<sup>22</sup>  
 54(59). *Buxus acunae* Borhidi & O.Muñiz  
 55(60). *Buxus aneura* Urb.  
 56(61). *Buxus arborea* Proctor  
 57(62). *Buxus bahamensis* Baker<sup>23</sup>  
 58(63). *Buxus baracoensis* Borhidi & O.Muñiz<sup>24</sup>  
 59(64). *Buxus bartlettii* Standl.  
 60(65). *Buxus bissei* Eg.Köhler  
 61(66). *Buxus braimbridgeorum* Eg.Köhler  
 62(67). *Buxus brevipes* (Müll.Arg.) Urb.<sup>25</sup>  
 63(68). *Buxus citrifolia* (Willd.) Spreng.<sup>26</sup>  
 64(69). *Buxus crassifolia* (Britton) Urb.<sup>27</sup>  
 65(70). *Buxus cristalensis* Eg. Köhler & P.A.González  
 66(71). *Buxus cubana* (A.Rich.) Baill.<sup>28</sup>  
 67(72). *Buxus cyclophylla* Floden & Shipunov, nom.nov.<sup>29</sup>

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<sup>15</sup> *Buxus calophylla* Pax; *Buxanthus hildebrandtii* Tiegh.; *Buxus pedicellata* (Tiegh.) Hutch.; *Buxanthus pedicellatus* Tiegh.

<sup>16</sup> *Buxella macowanii* Tiegh.; *Notobuxus macowanii* (Oliv.) E. Phillips

<sup>17</sup> *Buxella madagascarica* Tiegh.; *Notobuxus madagascarica* (Baill.) E. Phillips

<sup>18</sup> *Notobuxus natalensis* Oliv.

<sup>19</sup> *Notobuxus nyasica* (Hutch.) E.Phillips

<sup>20</sup> *Notobuxus obtusifolia* Mildbr.

<sup>21</sup> *Buxus angustifolia* G.E.Schatz & Lowry

<sup>22</sup> *Tricera acuminata* Griseb.

<sup>23</sup> *Tricera bahamensis* (Baker) Britton

<sup>24</sup> *Buxus shaferi* (Britton) Urb. p.p. *sensu* Eg.Köhler

<sup>25</sup> *Tricera brevipes* Britton

<sup>26</sup> *Tricera citrifolia* Willd.

<sup>27</sup> *Tricera crassifolia* Britton.

<sup>28</sup> *Tricera cubana* A.Rich.

<sup>29</sup> *Tricera rotundifolia* Britton; *Buxus rotundifolia* (Britton) Mathou, nom.illeg., non *Buxus rotundifolia* hort. ex K.Koch

- 68(73). *Buxus ekmanii* Urb.  
 69(74). *Buxus excisa* Urb.  
 70(75). *Buxus flaviramea* (Britton) Mathou<sup>30</sup>  
 71(76). *Buxus foliosa* (Britton) Urb.<sup>31</sup>  
 72(77). *Buxus glomerata* (Griseb.) Müll.Arg.<sup>32</sup>  
 73(78). *Buxus gonoclada* (Griseb.) Müll.Arg.<sup>33</sup>  
 74(79). *Buxus heterophylla* Urb.<sup>34</sup>  
 75(80). *Buxus historica* Borhidi & O.Muñiz<sup>35</sup>  
 76(81). *Buxus imbricata* Urb.  
 77(82). *Buxus jaucoensis* Eg.Köhler  
 78(83). *Buxus koehleri* P.A.González & Borsch  
 79(84). *Buxus laevigata* Spreng.  
 80(85). *Buxus lancifolia* Brandegee  
 81(86). *Buxus leivae* Eg.Köhler  
 82(87). *Buxus leonii* (Britton) Mathou<sup>36</sup>  
 83(88). *Buxus macrophylla* (Britton) Fawc. & Rendle<sup>37</sup>  
 84(89). *Buxus marginalis* (Britton) Urb.<sup>38</sup>  
 85(90). *Buxus mexicana* Brandegee  
 86(91). *Buxus moana* Alain  
 87(92). *Buxus moctezumae* Eg.Köhler, R.Fernald &  
     Zamudio  
 88(93). *Buxus muelleriana* Urb.<sup>39</sup>  
 89(94). *Buxus nipensis* Eg. Köhler & P.A.González  
 90(95). *Buxus obovata* Urb.<sup>40</sup>  
 91(96). *Buxus olivacea* Urb.  
 92(97). *Buxus pilosula* Urb.  
 93(98). *Buxus portoricensis* Alain  
 94(99). *Buxus pseudaneura* Eg.Köhler  
 95(100). *Buxus pubescens* Greenm.  
 96(101). *Buxus pulchella* Baill.  
 97(102). *Buxus purdieana* Baill.  
 98(103). *Buxus retusa* (Griseb.) Müll.Arg.<sup>41</sup>  
 99(104). *Buxus revoluta* (Britton) Mathou<sup>42</sup>

<sup>30</sup> *Tricera flaviramea* Britton; *Buxus gonoclada* (Griseb.) Müll.Arg. p.p. *sensu* Eg.Köhler

<sup>31</sup> *Tricera foliosa* Britton

<sup>32</sup> *Tricera glomerata* Griseb.

<sup>33</sup> *Tricera gonoclada* Griseb.

<sup>34</sup> *Buxus gonoclada* (Griseb.) Müll.Arg. p.p. *sensu* Eg.Köhler

<sup>35</sup> *Buxus retusa* (Griseb.) Müll.Arg. p.p. *sensu* Eg.Köhler

<sup>36</sup> *Tricera leonii* Britton; *Buxus wrightii* subsp. *leonii* (Britton) Eg.Köhler

<sup>37</sup> *Tricera macrophylla* Britton

<sup>38</sup> *Tricera marginalis* Britton

<sup>39</sup> *Tricera muelleriana* Britton

<sup>40</sup> *Buxus shaferi* (Britton) Urb. p.p. *sensu* Eg.Köhler

<sup>41</sup> *Tricera retusa* Griseb.

<sup>42</sup> *Tricera revoluta* Britton

- 100(105). *Buxus rheedioides* Urb.  
 101(106). *Buxus sclerophylla* Eg.Köhler  
 102(107). *Buxus serpentinicola* Eg.Köhler  
 103(108). *Buxus shaferi* (Britton) Urb.<sup>43</sup>  
 104(109). *Buxus subcolumnaris* Müll.Arg.  
 105(110). *Buxus triptera* Eg.Köhler  
 106(111). *Buxus vaccinioides* (Britton) Urb.<sup>44</sup>  
 107(112). *Buxus vahlii* Baill.  
 108(113). *Buxus wrightii* Müll.Arg.<sup>45</sup>  
 109(114). *Buxus yunquensis* Eg.Köhler

### Subfamilia *Pachysandroideae* Record & Garratt

#### Genus 4. SARCOCOCCA Lindl.

- 1(115). *Sarcococca balansae* Gagnep.  
 2(116). *Sarcococca bleddynii* J.M.H.Shaw & N. van Du  
 3(117). *Sarcococca confertiflora* Sealy  
 4(118). *Sarcococca confusa* Sealy  
 5(119). *Sarcococca coriacea* Sweet<sup>46</sup>  
 6(120). *Sarcococca hookeriana* Baill.<sup>47</sup>  
 7(121). *Sarcococca euphlebia* Merrill  
 8(122). *Sarcococca longifolia* M. Cheng & K.F. Wu  
 9(123). *Sarcococca longipetiolata* M. Cheng  
 10(124). *Sarcococca orientalis* C.Y. Wu  
 11(125). *Sarcococca philippinensis* Staph ex Sealy  
 12(126). *Sarcococca ruscifolia* Staph<sup>48</sup>  
 13(127). *Sarcococca saligna* (D.Don.) Müll.Arg.<sup>49</sup>  
 14(128). *Sarcococca wallichii* Staph  
 15(129). *Sarcococca zeylanica* Baill.<sup>50</sup>  
 16(130). *Sarcococca zollingeri* Baill.

#### Genus 5. SEALYA Floden & Shipunov, gen.nov.

- 1(131). *Sealya conzattii* (Standl). Floden & Shipunov,  
 comb.nov.<sup>51</sup>

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<sup>43</sup> *Tricerca shaferi* Britton

<sup>44</sup> *Tricerca vaccinioides* Britton

<sup>45</sup> *Tricerca microphylla* Griseb.

<sup>46</sup> *Sarcococca nepalensis* Royle; *Sarcococca pruniformis* Lindl. p.p.; *Sarcococca tonkinensis* Gagnep.; *Sarcococca trinervia* Wight; *Sarcococca vagans* Staph

<sup>47</sup> *Sarcococca humilis* Staph

<sup>48</sup> *Sarcococca pauciflora* C.Y.Wu

<sup>49</sup> *Sarcococca pruniformis* Lindl. p.p.

<sup>50</sup> *Sarcococca brevifolia* (Muell. Arg.) Staph ex Gamble

<sup>51</sup> *Sarcococca conzattii* (Standl.) I.M. Johnst.; *Buxus conzattii* Standl.

Genus 6. PACHYSANDRA Michx.

- 1(132). *Pachysandra terminalis* Siebold & Zucc. sed.m.
- 2(133). *Pachysandra axillaris* Franch.<sup>52</sup>
- 3(134). *Pachysandra procumbens* Michx.

Genus 7. STYLOCERAS Kunth ex A.Juss.

- 1(135). *Styloceras brokawii* A.H.Gentry & R.B.Foster
- 2(136). *Styloceras columnare* Müll.Arg.
- 3(137). *Styloceras connatum* Torrez & P. Jørg.
- 4(138). *Styloceras kunthianum* A. Juss.
- 5(139). *Styloceras laurifolium* (Willd.) Kunth
- 6(140). *Styloceras penninervium* A.H. Gentry & G.A. Aymard

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<sup>52</sup>*Pachysandra stylosa* Dunn

## \_shipunov\_foden\_et\_al\_buxineae

<b>Taxon name</b>	<b>Sample ID</b>	<b>Voucher (herbarium ID or similar)</b>
<i>Buxus acuminata</i>	B-201	Jongkind 9408 (PRE)
<i>Buxus acutata</i>	B-420	Breteler 13756 (MO-5759128)
<i>Buxus acutata</i>	B-421	De Wilde 9408 (MO-4310785)
<i>Buxus aneura</i>	B-118	Ekman 15176 (NY)
<i>Buxus arborea</i>	B-101	Gentry 28311 (NY-01381367)
<i>Buxus austroyunnanensis</i>	B-722	Zhou 619 (CAS-1153485)
<i>Buxus bahamensis</i>	B-1601	Correll 43786 (BRIT)
<i>Buxus bahamensis</i>	B-635	Webster 10423 (US-2432374)
<i>Buxus balearica</i>	B-622	Kennedy 865D (US-1862163)
<i>Buxus bartlettii</i>	B-716	Wendt 3672 (CAS-732187)
<i>Buxus benguillensis</i>	B-202	PRE-854763.0
<i>Buxus bissei</i>	B-510	Shafer 4277 (HUH)
<i>Buxus bodinieri</i>	B-1819	PE-01981551
<i>Buxus braimbridgeorum</i>	B-103	Shafer 8151 (NY-02150567)
<i>Buxus braimbridgeorum</i>	B-125	Shafer 8151 (NY-02150594)
<i>Buxus brevipes</i>	B-122	Acuna 17252 (NY-02150575)
<i>Buxus brevipes</i>	B-634	Brother Alain 2305 (US-2284282)
<i>Buxus calcarea</i>	B-426	Andriamihajarivo 507 (MO-6298213)
<i>Buxus capuronii</i>	B-425	Capuron 24212-SF (MO-5730160)
<i>Buxus cephalantha shantouensis</i>	B-1807	IBSC-0754341
<i>Buxus chaoanensis</i>	B-1808	IBSC-0345394
<i>Buxus cipolinica</i>	B-205	Birkinshaw 1399 (PRE)
<i>Buxus cipolinica</i>	B-206	Birkinshaw 1399 (PRE)
<i>Buxus citrifolia</i>	B-623	Bordenave 8253 (US-3523221)
<i>Buxus cochinchinensis</i>	B-1818	Larsen 31272 (PE-00044005)
<i>Buxus cochinchinensis</i>	B-1902	Shimizu 7957 (TI)
<i>Buxus cochinchinensis</i>	B-621	Pot 2488 (US-2589403)
<i>Buxus cochinchinensis</i>	B-809	Soejarto 14857 (F-2300811)
<i>Buxus cordata</i>	B-1602	Mwangoka 1274 (BRIT)
<i>Buxus cordata</i>	B-200	Mwasumbi 2505 (PRE)
<i>Buxus cordata</i>	B-422	Mwangoka 1274 (MO-5584102)
<i>Buxus crassifolia</i>	B-100	Sattler C732002 (NY-01381373)
<i>Buxus excisa</i>	B-114	Ekman 3809 (NY)

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Buxus flavidamea	B-107	Greuter s.n. (NY-01381374)
Buxus foliosa	B-106	Shafer 4073 (NY-02150593)
Buxus glomerata	B-631	Garcia 3618 (US-3325282)
Buxus glomerata	B-711	Ekman 9477 (UC-996186)
Buxus gonoclada	B-632	Morton 8778 (US-2284934)
Buxus gonoclada	B-803	Ekman 18585 (F-1474623)
Buxus hainanensis	B-717	Lau 5437 (CAS-728942)
Buxus harlandii	B-620	Hu 6560 (US-2697068)
Buxus henryi	B-718	Xiao 3357 (CAS-1148720)
Buxus heterophylla	B-119	Ekman 7555 (NY)
Buxus hildebrandtii	B-808	Burger 2978 (F-1632789)
Buxus humbertii	B-409	Ratovoson 1539 (MO-6426683)
Buxus ichagensis	B-1815	PE-00044079
Buxus ichagensis	B-1816	PE-00044075
Buxus imbricata	B-710	Ekman 15979 (UC-996190)
Buxus itremoensis	B-407	Shatz 3980 (MO-5814397)
Buxus itremoensis	B-408	Randrianaivo 154 (MO-5206210)
Buxus laevigata	B-123	Harris 10755 (NY-02150672)
Buxus lancifolia	B-713b	Purpus 5304 (UC-155266)
Buxus latistyla	B-1810	IBSC-0705548
Buxus latistyla	B-1814	PE-00044093
Buxus leonii	B-121	Brother Leon 4874 (NY)
Buxus linearifolia	B-1809	IBSC-0345454
Buxus liukiuensis	B-619	Elliott 609 (US-2594126)
Buxus macowanii	B-207	No voucher, cultivated, photo in repository
Buxus macowanii	B-612	Hardy 5337 (US-2999716)
Buxus macrocarpa	B-406	Rabe 83 (MO-6574040)
Buxus macrophylla	B-630	Proctor 11819 (US-2585569)
Buxus madagascarica	B-412	Razakamalala 2653 (MO-6426702)
Buxus madagascarica	B-413	Rabehevitra 1117 (MO-6039950)
Buxus madagascarica	B-611	Rabevohitra 3799 (US-3596205)
Buxus marginalis	B-629	Axelrod 10410 (US-3397787)
Buxus megistophylla	B-1901	Taam 535 (TI)
Buxus mexicana	B-712b	Purpus 2972 (UC-112959)

Buxus mexicana	B-723	Salinas 7524 (CAS-895786)
Buxus microphylla	B-618	Moran 4286 (US-2186325)
Buxus microphylla	B-704	Bartholomew 233 (UC-1491018)
Buxus moctezumae	B-724	Zamudio 11702 (CAS-1027047)
Buxus mollicula	B-503	Hu 13051 (HUH)
Buxus monticola	B-112	Razanatsima 123 (NY)
Buxus monticola	B-410	Capuron 23522-SF (MO-5727647)
Buxus monticola	B-411	Razanatsima 123 (MO-6053339)
Buxus moratii	B-418	De Block 2209 (MO-6301378)
Buxus moratii	B-610	Gillespie 4112 (US-3225197)
Buxus muelleriana	B-505	Brother Alain 5451 (HUH)
Buxus myrica	B-616	Wang 33332 (US-1670146)
Buxus myrica	B-706	Gressitt 1305 (UC-1352900)
Buxus nipensis	B-113	Shafer 3219 (NY-02150556)
Buxus nyasica	B-204	Chapman 8828 (PRE)
Buxus obovata	B-110	Ekman 15953 (NY-02150588)
Buxus obtusifolia	B-810	Faden 77/647 (F-2155753)
Buxus olivacea	B-709	Ekman 15218 (UC-996194)
Buxus olivacea	B-817	Ekman 4992 (F-1474625)
Buxus papillosa	B-617	Rechinger 29820 (US-2637743)
Buxus papillosa	B-705	Rechinger 29820 (UC-1376594)
Buxus pilosula	B-111	Brother Leon 19897 (NY-02150633)
Buxus portoricensis	B-816	Brother Alain 9811 (F-1618398)
Buxus pubescens	B-636	Ferris 5676 (US-1269103)
Buxus pubiramea	B-1805	IBSC-0345586
Buxus pulchella	B-109	Britton 4150 (NY-02150671)
Buxus purdieana	B-802	Harris 8964 (F-212020)
Buxus rabenantoandroi	B-414	Razakamalala 4530 (MO-6426685)
Buxus retusa	B-105	Abbott 18972 (NY-01381392)
Buxus retusa	B-502	Brother Alain 3668 (HUH)
Buxus revoluta	B-104	Berazain s.n. (NY-01381393)
Buxus rheedioides	B-117	Ekman 15175 (NY)
Buxus rolfei	B-614	Larsen 33973 (US-3376317)
Buxus rotundifolia	B-115	Shafer 4114 (NY)

<i>Buxus rugulosa</i>	B-721	Boufford 2884 (CAS-1011214)
<i>Buxus sempervirens</i>	B-003	No voucher, cultivated, photo in repository
<i>Buxus sempervirens hyrcana</i>	B-302	No voucher, photo in repository
<i>Buxus shaferi</i>	B-627	Brother Alain 4761 (US-2288281)
<i>Buxus sinica</i>	B-720	Yuan 1086 (CAS-846679)
<i>Buxus sirindhorniana</i>	B-500	van de Bult 664 (HUH)
<i>Buxus sp. celebica</i>	B-1817	PE-00044002
<i>Buxus sp. Philippines</i>	B-506	Reynoso 14113 (HUH)
<i>Buxus stenophylla</i>	B-1903	Hu 13026 (TI)
<i>Buxus subcolumnaris</i>	B-626	Howard 18878 (US-3001023)
<i>Buxus vaccinioides</i>	B-116	Shafer 4122 (NY)
<i>Buxus vaccinioides</i>	B-126	Shafer 4122 (NY)
<i>Buxus vahlii</i>	B-625	Little 21745 (US-2673754)
<i>Buxus vahlii</i>	B-807	Brother Alain 10722 (F-1618353)
<i>Buxus wallichiana</i>	B-615	Koelz 1831 (US-1605756)
<i>Didymelis integrifolia</i>	B-602	Schatz 2778 (US-3627925)
<i>Didymelis madagascariensis</i>	B-601	Rakotomazala 399 (US-3350972)
<i>Didymelis perrieri</i>	B-415	Razafimandribison 119 (MO-5617933)
<i>Didymelis sp. Toamasina</i>	B-417	Antilahimena 1529 (MO-6144923)
<i>Didymelis sp. Toamasina</i>	B-423	Antilahimena 2497a (MO-6177345)
<i>Pachysandra axillaris</i>	B-639	Hsien 1531 (US-1968483)
<i>Pachysandra axillaris</i>	B-703	Bonati s.n. (UC)
<i>Pachysandra axillaris</i>	B-714	Liu 15428 (CAS-943872)
<i>Pachysandra procumbens</i>	B-640	Kral 34 119 (US-2674034)
<i>Pachysandra terminalis</i>	B-005	No voucher, cultivated, photo in repository
<i>Sarcococca balansae</i>	B-303	No voucher, photo in repository
<i>Sarcococca balansae</i>	B-649	How 70661 (US-1675664)
<i>Sarcococca balansae</i>	B-811	How 70661 (F-779258)
<i>Sarcococca bleddynii</i>	foden3847	Foden s.n. (TENN)
<i>Sarcococca confusa</i>	B-713a	McClintock s.n. (CAS-582464)
<i>Sarcococca confuzzii</i>	B-651	Panero 5574 (US-3661245)
<i>Sarcococca confuzzii</i>	foden3834	Calzada 19727 (TENN)
<i>Sarcococca confuzzii</i>	foden3835	Calzada 19727 (TENN)
<i>Sarcococca confuzzii</i>	foden4073	Skutch 553 (TX)

<i>Sarcococca coriacea</i>	B-648	Nocolson 2712 (US-2571553)
<i>Sarcococca coriacea</i>	B-650	Bostick s.n. (US-29760003)
<i>Sarcococca euphlebia</i>	B-1801	IBSC-0757158
<i>Sarcococca hookeriana</i>	B-004	No voucher, cultivated, photo in repository
<i>Sarcococca hookeriana</i>	B-646	Bartholomew 1446 (US-3043590)
<i>Sarcococca longipetiolata</i>	B-1804	IBSC-0347445
<i>Sarcococca longipetiolata</i>	B-1812	PE-00055950
<i>Sarcococca orientalis</i>	B-1811	PE-01841407
<i>Sarcococca philippensis</i>	B-2001	Benguel 4645 (M)
<i>Sarcococca ruscifolia</i>	B-645	Rock 11598 (US-1333178)
<i>Sarcococca ruscifolia</i>	B-701	Bartholomew 1912 (UC-1491879)
<i>Sarcococca ruscifolia</i>	B-712a	Boufford 37718 (CAS-1103639)
<i>Sarcococca saligna</i>	B-644	Nasir 397 (US-2395364)
<i>Sarcococca saligna</i>	floden3971	Floden s.n. (TENN)
<i>Sarcococca</i> sp. Myanmar	B-3005	Hidetoshi 035324 (KYO)
<i>Sarcococca wallichii</i>	B-643	Bartholomew 688 (US-3068530)
<i>Sarcococca zeylanica</i>	B-642	Huber 874 (US-2868259)
<i>Sarcococca zeylanica</i>	B-647	Saldanha 10489 (US-2530653)
<i>Styloceras brokawii</i>	B-2101	Nee 52239 (SPF-168130)
<i>Styloceras brokawii</i>	B-2151	Nee 50191 (SP-350205)
<i>Styloceras brokawii</i>	B-606	Nee 39225 (US-3250366)
<i>Styloceras columnare</i>	B-1604	Lewis 881062 (BRIT)
<i>Styloceras columnare</i>	B-605	Cardenas 52 (US-2325019)
<i>Styloceras connatum</i>	B-401	Macia 3993 (MO-5905935)
<i>Styloceras connatum</i>	B-402	Maldonado 2688 (MO-5734632)
<i>Styloceras kunthianum</i>	B-2801	Altemirano 157 (QCA-26086)
<i>Styloceras kunthianum</i>	B-403	van der Werff 10955 (MO-3657438)
<i>Styloceras kunthianum</i>	B-404	Rubio 2224 (MO-4293135)
<i>Styloceras kunthianum</i>	B-818	Bonpland 9208 (F-1012222)
<i>Styloceras laurifolium</i>	B-1603	Lewis 37398 (BRIT)
<i>Styloceras laurifolium</i>	B-301	No voucher, cultivated, photo in repository
<i>Styloceras laurifolium</i>	B-400	Peyton 1083 (MO-3012831)
<i>Styloceras laurifolium</i>	B-603	Vargas 15578 (US-2438721)
<i>Styloceras laurifolium</i>	B-815	Young 4328 (F-1980936)

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<i>Styloceras penninervium</i>	B-427	Smith 3206 (MO-3395324)
<i>Styloceras</i> sp. Ecuador	B-604	Clark 8910 (US-3500727)
<i>Styloceras</i> sp. Peru Oxapampa	B-2202	Valenzuela 13978 (USM-252811)
<i>Trochodendron aralioides</i>	B-001	No voucher, cultivated, photo in repository
<i>Trochodendron aralioides</i>	B-002	No voucher, cultivated, photo in repository

<b>Region</b>	<b>Sequenced fragment</b>
Gabon	rbcl
Ivory Coast	rbcl, trnl
Gabon	rbcl
Cuba	rbcl, trnl
Jamaica	its, rbcl, trnl
Yunnan	its, rbcl, trnl
Bahamas	rbcl
Bahamas	trnl
Mallorca	its
Mexico	rbcl, trnl
Angola	its, rbcl, trnl
Cuba	rbcl
Guanxi	its, rbcl, trnl
Cuba	trnl
Cuba	rbcl, trnl
Cuba	its, rbcl, trnl
Cuba	rbcl
Madagascar	its, rbcl, trnl
Madagascar	its, rbcl, trnl
Guangdong	its, rbcl, trnl
Guangdong	its, rbcl, trnl
Madagascar	rbcl, trnl
Madagascar	its, rbcl, trnl
Suriname	rbcl, trnl
Thailand	rbcl
Thailand	rbcl
Thailand	rbcl, trnl
Vietnam	rbcl
Tanzania	rbcl, trnl
Tanzania	rbcl, trnl
Tanzania	rbcl, trnl
Cuba	its, rbcl, trnl
Cuba	rbcl, trnl

Cuba	rbcl, trnl
Cuba	rbcl, trnl
Hispaniola	its, rbcl, trnl
Cuba	rbcl, trnl
Cuba	trnl
Cuba	its, rbcl, trnl
Hainan	rbcl, trnl
Hong Kong	rbcl
Hunan	rbcl, trnl
Cuba	trnl
Ethiopia	rbcl
Madagascar	its, rbcl, trnl
Hubei	its, rbcl
Shaanxi	its, rbcl
Cuba	its, rbcl, trnl
Madagascar	its, rbcl, trnl
Madagascar	its, rbcl, trnl
Cuba	rbcl, trnl
Mexico	its, rbcl, trnl
Guanxi	its, rbcl
Guanxi	its, rbcl, trnl
Cuba	its, rbcl, trnl
Guanxi	rbcl
Okinawa	its, rbcl
Cape	its, rbcl, trnl
South Africa	rbcl, trnl
Madagascar	its, rbcl, trnl
Jamaica	rbcl, trnl
Madagascar	its, rbcl
Madagascar	its, rbcl, trnl
Madagascar	its, rbcl
Cuba	rbcl, trnl
Guangdong	its, rbcl, trnl
Mexico	its, rbcl, trnl

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Mexico	its, rbcl, trnl
Korea	rbcl
Hubei	rbcl, trnl
Mexico	rbcl, trnl
Hong Kong	its, rbcl, trnl
Madagascar	its, rbcl, trnl
Madagascar	rbcl
Madagascar	its, rbcl, trnl
Madagascar	rbcl, trnl
Madagascar	its, rbcl, trnl
Cuba	rbcl
Hainan	rbcl, trnl
Hainan	its, rbcl, trnl
Cuba	rbcl
Malawi	its, rbcl
Cuba	trnl
Kenya	rbcl, trnl
Cuba	rbcl
Cuba	rbcl
Pakistan	its, trnl
Pakistan	its, trnl
Cuba	its, rbcl, trnl
Puerto Rico	trnl
Mexico	its, rbcl, trnl
Hainan	rbcl
Jamaica	rbcl
Jamaica	rbcl, trnl
Madagascar	its, rbcl
Cuba	rbcl, trnl
Cuba	trnl
Cuba	its, rbcl, trnl
Cuba	its, rbcl, trnl
Thailand	its, rbcl, trnl
Cuba	rbcl, trnl

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Sichuan	its, rbcl, trnl
USA	its
Azerbaijan	its, rbcl, trnl
Cuba	its, rbcl, trnl
Gansu	its, rbcl, trnl
Thailand	its, rbcl, trnl
China	rbcl
Sibuyan	its, rbcl
Hong Kong	its, rbcl, trnl
Martinique	its, rbcl, trnl
Cuba	rbcl
Cuba	its, rbcl, trnl
Puerto Rico	its, trnl
Puerto Rico	rbcl, trnl
Himachal Pradesh	its, rbcl, trnl
Madagascar	rbcl
Madagascar	its, rbcl, trnl
Madagascar	its, rbcl, trnl
Madagascar	rbcl
Madagascar	its
Sichuan	its, trnl
Yunnan	its
Sichuan	its, trnl
Tennessee	its, trnl
USA	its, rbcl
Vietnam	its, rbcl
Hainan	rbcl, trnl
Hainan	rbcl
Vietnam	its, rbcl, trnl
Cultivated	its, rbcl, trnl
Mexico	rbcl, trnl
Mexico	rbcl, trnl
Mexico	its, trnl
Guatemala	its, trnl

Nepal	its, rbcl
Cultivated	its, rbcl, trnl
Hainan	its, rbcl, trnl
USA	its, rbcl, trnl
Yunnan	its, rbcl, trnl
Guangdong	rbcl
Hunan	its, rbcl, trnl
Guanxi	its, rbcl, trnl
Luzon	rbcl, trnl
Yunnan	its, rbcl, trnl
Hubei	its, rbcl, trnl
Gansu	its, rbcl, trnl
Pakistan	its, rbcl, trnl
NA	its, trnl
Myanmar	its, rbcl
Yunnan	its, rbcl, trnl
Ceylon	its, rbcl
Tamil Nadu	its, rbcl, trnl
Bolivia	rbcl, trnl
Bolivia	rbcl
Bolivia	rbcl, trnl
Bolivia	its, rbcl
Bolivia	its, rbcl
Bolivia	its
Ecuador	rbcl, trnl
Ecuador	its, rbcl
Ecuador	trnl
Ecuador	its, trnl
Bolivia	rbcl, trnl
Colombia	its, trnl
Peru	its
Peru	its, rbcl, trnl
Peru	trnl

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Peru	its, rbcl, trnl
Ecuador	rbcl
Peru	rbcl
USA	its
USA	its

**buxineae\_dna\_genbank\_ids.txt**

<b>Sequence ID</b>	<b>Fragment</b>	<b>GenBank ID</b>
B-101	its	MN537041
B-722	its	MN537042
B-622	its	MN537043
B-202	its	MN537044
B-1819	its	MN537045
B-122	its	MN537046
B-426	its	MN537047
B-425	its	MN537048
B-1807	its	MN537049
B-1808	its	MN537050
B-100	its	MN537051
B-803	its	MN537052
B-409	its	MN537053
B-1815	its	MN537054
B-407	its	MN537055
B-713b	its	MN537056
B-1810	its	MN537057
B-121	its	MN537058
B-619	its	MN537059
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buxineae\_dna\_genbank\_ids.txt

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B-627	its	MN537080
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B-626	its	MN537085
B-126	its	MN537086
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B-1812	its	MN537102
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buxineae\_dna\_genbank\_ids.txt

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B-646	its	MN537125
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B-104	rbcl	MN579952
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buxineae\_dna\_genbank\_ids.txt

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B-1814	rbcl	MN579983
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B-1818	rbcl	MN579987
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B-1901	rbcl	MN579989
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B-1903	rbcl	MN579991
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buxineae\_dna\_genbank\_ids.txt

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buxineae\_dna\_genbank\_ids.txt

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B-619	rbcl	MN580045
B-620	rbcl	MN580046
B-621	rbcl	MN580047
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B-704	rbcl	MN580067
B-706	rbcl	MN580068
B-709	rbcl	MN580069

buxineae\_dna\_genbank\_ids.txt

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B-712a	rbcl	MN580072
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B-713a	rbcl	MN580074
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B-717	rbcl	MN580077
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buxineae\_dna\_genbank\_ids.txt

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B-107	trnl	MN549179
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B-631	trnl	MN549181
B-632	trnl	MN549182
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B-112	trnl	MN549203
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buxineae\_dna\_genbank\_ids.txt

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B-810	trnl	MN549208
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B-111	trnl	MN549210
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B-636	trnl	MN549212
B-802	trnl	MN549213
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B-104	trnl	MN549215
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B-614	trnl	MN549217
B-115	trnl	MN549218
B-721	trnl	MN549219
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B-625	trnl	MN549227
B-615	trnl	MN549228
B-601	trnl	MN549229
B-415	trnl	MN549230
B-639	trnl	MN549231
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B-649	trnl	MN549233
floden3847	trnl	MN549234
B-713a	trnl	MN549235
B-651	trnl	MN549236
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B-1811	trnl	MN549240
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buxineae\_dna\_genbank\_ids.txt

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B-643	trnl	MN549246
B-647	trnl	MN549247
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B-404	trnl	MN549251
B-301	trnl	MN549252
B-427	trnl	MN549253
B-125	trnl	MN549254
B-206	trnl	MN549255
B-1602	trnl	MN549256
B-711	trnl	MN549257
B-803	trnl	MN549258
B-408	trnl	MN549259
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B-603	trnl	MN549272
floden3835	trnl	MN549273
B-712a	trnl	MN549274
B-815	trnl	MN549275
floden4073	trnl	MN549276
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## 04\_buxaceae\_used\_genbank\_ids.tx

<b>GenBank ID</b>	<b>Species</b>	<b>Fragment</b>
JF978888	<i>Tetracentron sinense</i>	its
AF245416	<i>Sarcococca confertiflora</i>	its
slx077	<i>Hapanthus hazlettii</i>	its
AF245425	<i>Buxus natalensis</i>	its
AF245412	<i>Buxus microphylla</i>	its
AF245415	<i>Buxus hildebrandtii</i>	its
AF245409	<i>Buxus henryi</i>	its
AF245410	<i>Buxus harlandii</i>	its
AF245433	<i>Buxus citrifolia</i>	its
AF245434	<i>Buxus acutata</i>	its
GQ998807	<i>Trochodendron aralioides</i>	matk
AM396504	<i>Tetracentron sinense</i>	matk
LN877480	<i>Styloceras laurifolium</i>	matk
LN877483	<i>Sarcococca saligna</i>	matk
LN877488	<i>Sarcococca hookeriana</i>	matk
LN877481	<i>Sarcococca connzattii</i>	matk
LN877482	<i>Sarcococca confusa</i>	matk
AF542581	<i>Pachysandra terminalis</i>	matk
GU266592	<i>Pachysandra procumbens</i>	matk
KX526614	<i>Pachysandra axillaris</i>	matk
LN877495	<i>Hapanthus hazlettii</i>	matk
DQ401354	<i>Didymeles perrieri</i>	matk
LN877440	<i>Buxus yunquensis</i>	matk
LN877502	<i>Buxus wrightii</i>	matk
LN877443	<i>Buxus vahlii</i>	matk
LN877458	<i>Buxus triptera</i>	matk
KP088983	<i>Buxus sinica</i>	matk
LN877400	<i>Buxus shaferi</i>	matk
LN877419	<i>Buxus serpentinicola</i>	matk
LN877449	<i>Buxus sempervirens</i>	matk
LN877418	<i>Buxus sclerophylla</i>	matk
LN877399	<i>Buxus rotundifolia</i>	matk
LN877471	<i>Buxus rheedioides</i>	matk
LN877431	<i>Buxus revoluta</i>	matk
LN877408	<i>Buxus retusa</i>	matk
LN877496	<i>Buxus pubescens</i>	matk
LN877417	<i>Buxus pseudaneura</i>	matk
LN877484	<i>Buxus portoricensis</i>	matk
LN877416	<i>Buxus pilosula</i>	matk
MG946997	<i>Buxus papillosa</i>	matk
LN877415	<i>Buxus olivacea</i>	matk
HG004438	<i>Buxus nipensis</i>	matk
LN877461	<i>Buxus natalensis</i>	matk

04\_buxaceae\_used\_genbank\_ids.tx

AF186395	Buxus muelleriana	matk
LN877494	Buxus moratii	matk
LN877412	Buxus moctezumae	matk
LN877411	Buxus moana	matk
LN877447	Buxus microphylla	matk
LN877442	Buxus mexicana	matk
LN877410	Buxus marginalis	matk
LN877498	Buxus madagascarica	matk
LN877460	Buxus macowanii	matk
LN877413	Buxus leivae	matk
HG004433	Buxus koehleri	matk
LN877409	Buxus jaucoensis	matk
LN877441	Buxus imbricata	matk
LN877462	Buxus hildebrandtii	matk
AF186398	Buxus harlandii	matk
LN877426	Buxus gonoclada	matk
LN877439	Buxus glomerata	matk
LN877407	Buxus foliosa	matk
LN877406	Buxus excisa	matk
LN877405	Buxus ekmanii	matk
HG004434	Buxus cristalensis	matk
LN877398	Buxus crassifolia	matk
LN877497	Buxus citrifolia	matk
LN877486	Buxus brevipes	matk
LN877453	Buxus braimbridgeorum	matk
LN877404	Buxus bissei	matk
LN877459	Buxus benguellensis	matk
LN877403	Buxus bartlettii	matk
LN877446	Buxus balearica	matk
LN877421	Buxus bahamensis	matk
LN877444	Buxus arborea	matk
LN877397	Buxus aneura	matk
LN877402	Buxus acunae	matk
AY590833	Trochodendron aralioides	petd
AM396539	Tetracentron sinense	petd
LN877590	Styloceras laurifolium	petd
LN877593	Sarcococca saligna	petd
LN877598	Sarcococca hookeriana	petd
LN877591	Sarcococca confuzii	petd
LN877592	Sarcococca confusa	petd
AM396541	Pachysandra terminalis	petd
LN877605	Haploanthus hazlettii	petd
AM396540	Didymelis integrifolia	petd
LN877548	Buxus yunquensis	petd

04\_buxaceae\_used\_genbank\_ids.tx

LN877612	Buxus wrightii	petd
LN877595	Buxus vahlii	petd
LN877567	Buxus triptera	petd
LN877544	Buxus shaferi	petd
LN877527	Buxus serpentinicola	petd
AY590832	Buxus sempervirens	petd
LN877526	Buxus sclerophylla	petd
LN877579	Buxus rotundifolia	petd
LN877537	Buxus rheedioides	petd
LN877541	Buxus revoluta	petd
LN877578	Buxus retusa	petd
LN877606	Buxus pubescens	petd
LN877524	Buxus pseudaneura	petd
LN877594	Buxus portoricensis	petd
LN877575	Buxus pilosula	petd
LN877577	Buxus olivacea	petd
LN877580	Buxus nipensis	petd
LN877570	Buxus natalensis	petd
LN877604	Buxus moratii	petd
LN877519	Buxus moctezumae	petd
LN877518	Buxus moana	petd
LN877557	Buxus microphylla	petd
LN877551	Buxus mexicana	petd
LN877517	Buxus marginalis	petd
LN877608	Buxus madagascarica	petd
LN877569	Buxus macowanii	petd
LN877520	Buxus leivae	petd
LN877550	Buxus koehleri	petd
LN877516	Buxus jaucoensis	petd
LN877549	Buxus imbricata	petd
LN877571	Buxus hildebrandtii	petd
LN877546	Buxus gonoclada	petd
LN877534	Buxus glomerata	petd
LN877514	Buxus foliosa	petd
LN877513	Buxus excisa	petd
LN877565	Buxus ekmanii	petd
LN877525	Buxus cristalensis	petd
LN877505	Buxus crassifolia	petd
LN877607	Buxus citrifolia	petd
LN877596	Buxus brevipes	petd
LN877562	Buxus braimbridgeorum	petd
LN877511	Buxus bissei	petd
LN877568	Buxus benguellensis	petd
LN877510	Buxus bartlettii	petd

04\_buxaceae\_used\_genbank\_ids.tx

LN877555	Buxus balearica	petd
LN877529	Buxus bahamensis	petd
LN877553	Buxus arborea	petd
LN877504	Buxus aneura	petd
LN877509	Buxus acunae	petd
LN877508	Buxus acuminata	petd
GQ998840	Trochodendron aralioides	rbcl
L12668	Tetracentron sinense	rbcl
AF061993	Pachysandra procumbens	rbcl
AF203485	Pachysandra axillaris	rbcl
HQ634681	Haptanthus hazlettii	rbcl
DQ182333	Buxus sempervirens	rbcl
KJ082152	Buxus portoricensis	rbcl
MG946885	Buxus papillosa	rbcl
MG946906	Buxus natalensis	rbcl
HE963365	Buxus balearica	rbcl
AY145360	Trochodendron aralioides	trnl
AM397165	Tetracentron sinense	trnl
AM397167	Pachysandra terminalis	trnl
slx077	Haptanthus hazlettii	trnl
AM397166	Didymelis integrifolia	trnl
LN877657	Buxus yunquensis	trnl
LN877719	Buxus wrightii	trnl
LN877675	Buxus triptera	trnl
LN877636	Buxus serpentinicola	trnl
AY145357	Buxus sempervirens	trnl
LN877635	Buxus sclerophylla	trnl
LN877634	Buxus pseudaneura	trnl
LN877632	Buxus olivacea	trnl
HG004431	Buxus nipensis	trnl
LN877678	Buxus natalensis	trnl
LN877628	Buxus moana	trnl
AB817516	Buxus liukiuensis	trnl
LN877630	Buxus leivae	trnl
HG004428	Buxus koehleri	trnl
LN877626	Buxus jaucoensis	trnl
LN877679	Buxus hildebrandtii	trnl
AF200930	Buxus harlandii	trnl
LN877622	Buxus ekmanii	trnl
HG004429	Buxus cristalensis	trnl
LN877621	Buxus bissei	trnl
LN877663	Buxus balearica	trnl
LN877619	Buxus acunae	trnl
LN877618	Buxus acuminata	trnl