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

Running title: Phylogeny of Buxaceae

Alexey Shipunov¹, Hye Ji Lee¹, Jinhee Choi¹, Kyle Pay¹, Sarah DeSpiegelaire¹, Aaron Floden²

¹Minot State University, Biology, 500 University Ave, Minot, ND

²Missouri Botanical Garden Herbarium, Monsanto Center, 4344 Shaw Blvd, St. Louis, MO 63110

Acknowledgements

Our research was supported by North Dakota INBRE and by the Department of Biology of Minot State

University. Some of the sequences are results of collaboration with the Barcoding of Life project. We are grateful

to the curators of all herbaria provided us with samples, namely US, NY, HUH, MO, CAS, UC, JEPS, K, F,

BRIT, B, BO, IBSC, MICH, MO, NY, PE, TI, SPF, SP, USM, PRE, NBG, SAM. AF thanks Dr. Schilling for his

encouragement and allowance of taking on extracurricular projects into my many interests while working

towards my Ph.D., Dr. Jose Panero for sending the duplicate specimens that instigated part of this project, Joe

May and Veronica Brown at the UT Genomics Core, Kelly and Sue at Far Reaches Farm for an additional

Sarcococca wallichii, Dr. P.D. González Gutiérrez for discussion of his thesis work of Neotropical Buxus, and

Dr. T. Mitchell and B. Wynn-Jones for support in Vietnam. AS thanks Dr. Hidetoshi Nagamasu (University of

Kyoto) and Ms. Chikako Hasekura (Tokyo University of Agriculture) for the help with Japanese plants, and

Ekaterina Shipunova, Maxim Nuraliev (Moscow State University), Polina Volkova (Papanin Institute for

Biology of Inland Waters, Russia), Maria Kuzmina (University of Guelph), and Subdirección Científica, Jardín

Botánico de Bogotá José Celestino Mutis (Bogota, Colombia) for help with obtaining and sequencing samples.

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. *Didymeles*, 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 *Didymeles* from Madagascar.

Keywords: Buxaceae, Haptanthus, Didymeles, 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 *Didymeles* 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 steroidal 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 *Didymeles* (Shipunov & Shipunova, 2010; Oskolski & al., 2015).

Geographically, *Didymeles* 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 *Didymeles* 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 *Sarococca* 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. conzattii* (Standl.) I.M. Johnst., 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 NUCLEOSPIN 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 Ripeline (Shipunov, 2020), the R-based DNA sequence analysis pipeline for databasing, sequence analysis, and phylogeny estimation. Ripeline 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, Ripeline is capable of using morphological characters, perform nearest neighbor imputation of missed sequences, and producing super-alignments (Ashkenazy & al., 2018). The Ripeline is available from the primary author's Github: https://github.com/ashipunov/Ripeline. Within Ripeline, 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 Ripeline 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 Ripeline, 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 phylogenic 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.

Didymeles and Haptanthus

Didymeles 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 *Didymeles* + *Haptanthus* grade, was supported well (BPP > 96%) on CP and OI trees. A morphologically distinct (Fig. 7) sample of *Didymeles* from Toamasina (Madagascar) recovered as a first branch in the *Didymeles* 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. flaviramea* (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. conzattii* 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 conzattii 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 conzattii* 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 at 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

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

corresponds with the more recent review of Tianlu & Brückner (2008).

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 is 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 conzattii* (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 is 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 the 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

Didymeles toamasinae Floden et Shipunov, sp.nov.

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

TYPE:—Toamasina. Fivondronana: Maroantsetra. Commume: Antisrasbesahatany. Fokontany: Anjiahely. Beanivona forest. Collected with Pascal, Ramaroson, Saside and David. 15°23'56"S 045°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 $3-5 \times 1.5-3$ 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

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: "Kichijisô, 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 ... *Didymeles* (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 ... *Haptanthus* (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
 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.

Literature cited

Ashkenazy, H., Sela, I., Levy Karin, E., Landan, G. & Pupko, T., 2018. Multiple sequence alignment averaging improves phylogeny reconstruction. *Syst. Biol.* 68: 117–130.

Backer, C.A. & Bakhuizen van den Brink, R.C. 1965. Flora of Java 1: 646-647.

Baillon, H. 1859. Monographie des Buxacées et des Stylocérées. Paris, Victor Masson.

Batchelor, J. & Miyabe, K. 1893. Ainu economic plants. Yokohama, 1893.

Beilschmid, C.T. 1833. J. Lindley's characteres distinctivi oder Hauptkennzeichen der natürlichen Pflanzenfamilien. *Flora*. 16: 49–112

Bejarano, L. 2015. Reporte y caracterización de poblaciones, fenología y descripción del fruto de *Haptanthus* hazlettii, una especie endémica de Honduras. *Tatascán*. 25: 31–41.

Borchsenius, F. 2009. FastGap 1.2. Department of Biosciences, Aarhus University, Denmark. URL: http://www.aubot.dk/FastGap_home.htm (accessed Aug 27 2019).

Boufford, D.E. & Xiang, Q.Y. 1992. Pachysandra (Buxaceae) reexamined. Bot. Bull. Acad. Sinica. 33: 201–207.

Campbell, V., Legendre, P. & Lapointe, F.-J. 2011. The performance of the Congruence Among Distance Matrices (CADM) test in phylogenetic analysis. *BMC Evol. Biol.* 11: 64.

Castilho, R.O., Bulhões, A.A.D.S. & Kaplan, M.A.C. 1999. Controversy in Buxales systematic positioning. *Nord. J. Bot.* 19: 541–546.

Channell, R.B. & Wood, C.E. 1987. The Buxaceae in the southeastern United States. *J. Arnold Arb*. 68: 241–257.

Choi, J., Lee, H. & Shipunov, A. 2015. All that is gold does not glitter? Age, taxonomy, and ancient plant DNA quality. *PeerJ*. 3:e1087.

Dirr M.A. & Alexander J.H. 1979. Allegheny Pachysandra. Arnoldia 39: 16–21.

Doust, A.N. & Stevens, P.F. 2005. A reinterpretation of the staminate flowers of *Haptanthus*. *Syst. Bot.* 30: 779–785.

Dumortier, B.C. 1822. Commentationes Botanicae. P. 54.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32(5): 1792–1797.

Friis, I. 1989. A synopsis of the Buxaceae in Africa south of the Sahara. Kew Bull. 44: 293–299.

Funk, V.A., Edwards, R. & Sterling, K. 2018. The problem with(out) vouchers. *Taxon* 67: 3–5.

Gentry, A.H. & Aymard, G. 1993. A new species of Styloceras (Buxaceae) from Peru. Novon. 3: 142–144.

Gentry, A.H. & Foster R. 1981. A new Peruvian *Styloceras* (Buxaceae): discovery of a phytogeographical missing link. *Ann. Missouri Bot. Gard.* 68: 122–124.

Goldberg, A. & Alden H.A. 2005. Taxonomy of *Haptanthus* Goldberg & C. Nelson. Syst. Bot. 30: 773–778.

Goldberg, A. & Nelson, C.S. 1989. *Haptanthus*, a new dicotyledonous genus from Honduras. *Syst. Bot.* 14: 16–19.

Gray, A. 1846. Analogy between the flora of Japan and that of the United States. Am. J. Sci. ser. 2, 2:135–136.

Gray, J. & Sohma, K. 1964. Fossil *Pachysandra* from western America with a comparative study of pollen in *Pachysandra* and *Sarcococca*. *Am. J. Sci.* 262: 1159–1197.

Gutiérrez P.A.G. 2014. Evolution and biogeography of *Buxus* L.(Buxaceae) in Cuba and the Caribbean. Dissertation. Berlin: Freie Universität.

Gutiérrez, P.A.G. & Borsch, T. 2013. New species of *Buxus* (Buxaceae) from northeastern Cuba based on morphological and molecular characters, including some comments on molecular diagnosis. *Willdenowia*. 43: 125–137.

Hardman, R. 1987. Recent developments in our knowledge of steroids. *Planta Medica* 53: 233-238.

Hatusima, S. 1942. A revision of the Asiatic *Buxus*. *Journal of the Faculty of Agriculture, Kyushu University* 6: 261–342.

Hazlett, D.L. 2016. Especies taladas, volumen de madera extraído y un árbol endémico raro (*Haptanthus*) de un Bosque Hondureño de Tierras Bajas. *Ceiba*. 53: 81–94.

Heibl, C. 2008. IPS: R language tree plotting tools and interfaces to diverse phylogenetic software packages. URL: https://github.com/heibl/ips (accessed Aug 27 2019).

Reveal, J.L. & Hoogland, R.D. 1990. Validation of five family names in the Magnoliophyta. *Bulletin du Muséum national d'histoire naturelle. Section B, Adansonia* 12: 205–208.

Huelsenbeck, J.P., & Ronquist, R. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Jarvis, C.E. 1989. A review of the family Buxaceae Dumortier. Pp. 274–278 in: Crane P.R., Blackmore S. (eds). *Evolution, systematics, and fossil history of the Hamamelidae*. Clarendon Press, Oxford.

Jiao, Z. & Li, J. 2009. Phylogenetics and biogeography of eastern Asian–North American disjunct genus *Pachysandra* (Buxaceae) inferred from nucleotide sequences. *J Syst. Evol.* 47: 191–201.

Johnston, I.M. 1938. Some undescribed species from Mexico and Guatemala. J Arnold Arbor. 19: 117–128.

Johnston, I.M. 1939 New phanerogams from Mexico. J Arnold Arbor. 20: 234–240.

Köhler, E. & Brückner, P. 1982. Die pollenmorphologie der afrikanischen *Buxus*-und *Notobuxus*-arten (Buxaceae) und ihre systematische bedeutung. Grana, 21: 71–82.

Köhler, E. & Brückner, P. 1989. The genus *Buxus* (Buxaceae): aspects of its differentiation in space and time. *Plant Syst. Evol.* 162: 207–283.

Köhler, E. 1994. Parallel evolution of pollen characters in the genus *Buxus* L.(Buxaceae). *Acta Bot Gallica*. 141: 223–232.

Köhler, E. 1998. Weitere neue Buxus- Arten der Flora von Cuba. Feddes Repert. 109: 351–363.

Köhler, E. 2004. Buxaceae. Pp. 70–72 in: Smith, N., Mori, S.A., Henderson, A., Stevenson, D.W. & Head, S.W. (eds.). *Flowering Plants of the Neotropics*. Princeton University Press, Princeton.

Köhler, E. 2006. Three new Buxus species (Buxaceae) from eastern Cuba. Willdenowia. 36: 479–489.

Köhler, E. 2007. Buxales. Buxaceae. Didymelaceae. In: K. Kubitzki (ed.). *Flowering Plants: Eudicots. IX*. Berlin, Heidelberg: Springer.

Köhler, E. 2009.Neotropical Buxaceae. In: Milliken, W., Klitgård, B. & Baracat, A. 2009—. Neotropikey—Interactive key and information resources for flowering plants of the Neotropics. URL: http://www.kew.org/science/tropamerica/neotropikey/families/Buxaceae.htm (accessed 29 Aug 2019).

Köhler, E. 2014. Buxaceae. Flora de la Republica de Cuba. 19: 1–124.

Kunth, K.S. *Styloceras* In: Jussieu, A. 1824. *De Euphorbiacearum Generibus medisque earumdem viribus tentamen*. Paris 17: 53.

Kuzmina, M., Ivanova, N. 2011. Primer sets for plants and fungi. URL: http://ccdb.ca/site/wp-content/uploads/2016/09/CCDB_PrimerSets-Plants.pdf (accessed 29 Aug 2019).

Larson, P.D. 1996. Boxwood: its history, cultivation, propagation and descriptions. Foliar Press, Boyce, VA.

Larsson, A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. *Bioinformatics*. 30: 3276–3278.

Leandri, J. 1937. Sur 1'aire et la position systematique du genre malgache *Didymeles* Thouars. Annales des sciences naturelles. *Botanique*. Series 10. 19: 304–318.

Lindley J. 1826 Botanical Register; Consisting of Coloured Figures of Exotic Plants Cultivated in British Gardens; with their History and Mode of Treatment. Vol. 12. London

Linnaeus C. 1753. Species Plantarum. Holmiae.

Madagascar Catalogue. 2018. Catalogue of the Vascular Plants of Madagascar. Missouri Botanical Garden, St. Louis, U.S.A. & Antananarivo, Madagascar. URL: http://www.tropicos.org/Project/Madagascar. (Accessed Aug 29 2019)

Mathou, Th. 1940. Recherches sur le famille des Buxacées. Douladoure, Toulouse.

Michaux, A. 1803. Flora Boreali-Americana. Vol. 2. Caroli Crapelet, Paris.

Min Tianlu & Brückner, P. 2008. Buxaceae. In: Flora of China. 11: 321–332.

Nelson, C.S. 2001. Plantas descritas originalmente de Honduras y sus nomenclaturas equivalentes actuales. *Ceiba*. 42: 1–71.

Nixon, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics, 15: 407–414.

Oliver, D. 1882. *Notobuxus natalensis. Hooker's Icones Plantarum*; or Figures, with brief Descriptive Characters and Remarks of New or Rare Plants. 4: 78.

Oskolski, A., von Balthazar, M., Staedler, Y.M. & Shipunov, A.B. 2015. Inflorescence and floral morphology of *Haptanthus hazlettii* (Buxaceae, Buxales). *Bot. J. Linn. Soc.* 179: 190–200.

Paradis, E., Claude, J. & Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289–290.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/ (Accessed Aug 30, 2019)

Raven, P.H. & Axelrod, D.I. 1974. Angiosperm biogeography and past continental movements. *Ann. Missouri Bot. Gard.* 61: 539–673.

Record S.J. & Garratt G.A. Boxwoods. Yale University School of Forestry Bulletin. 14.

Reveal, J.L. 2011. Indices nominum supragenericorum plantarum vascularium. Alphabetical listing by genera of validly published suprageneric names. URL: http://www.plantsystematics.org/reveal/pbio/fam/allspgnames.html (Accessed Aug 30, 2019)

Reveal, J.L. 2012. An outline of a classification scheme for extant flowering plants. *Phytoneuron*. 37: 1–221.

Robbins, H.C. 1968. The genus Pachysandra (Buxaceae). Sida. 3: 211–248.

Rokas, A. & Carroll, S.B. 2005. More genes or more taxa? The relative contribution of gene number and taxon number to phylogenetic accuracy. *Molecular Biology and Evolution*. 22: 1337–1344.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*. 19: 1572–1574.

Rossello, J.A., Lazaro, A., Cosín, R., Molins, A. 2007. A phylogeographic split in *Buxus balearica* (Buxaceae) as evidenced by nuclear ribosomal markers: when ITS paralogues are welcome. *Journal of Molecular Evolution*. 64: 143–157.

Samarakoon, T., Wang, S.Y. & Alford, M.H., 2013. Enhancing PCR amplification of DNA from recalcitrant plant specimens using a trehalose- based additive. *Applications in Plant Sciences*. 1: 1200236.

Sauget, J.S. & Liogier, E.E. 1974. Buxaceae. Flora de Cuba. 2:140–146.

Schatz, G.E. & Lowry II, P.P. 2002. A synoptic revision of the genus *Buxus* L.(Buxaceae) in Madagascar and the Comoro Islands. *Adansonia*. 3: 7–19.

Schliep, K.P. 2011. phangorn: phylogenetic analysis in R. Bioinformatics. 27: 592–593.

Schreber, J.C.D. 1797. Tricera. In: Swartz O. Flora Indiae Occidentalis. 1: 331–334.

Sealy, J.R. 1986. A revision of the genus Sarcococca (Buxaceae). Bot. J. Linn. Soc. 92: 117–159.

Seifert, K. A., Crous P.W. & Frisvad J.C. 2008. Correcting the impact factors of taxonomic journals by appropriate citation of taxonomy (ACT). *Persoonia* 20: 105.

Shaw, J. 2011. New introductions from northern Vietnam. *Plantsman*. 10: 232–237.

Shipunov, A. & Shipunova E. 2011. *Haptanthus* story: Rediscovery of enigmatic flowering plant from Honduras. *Am. J. Bot.*. 98: 761–763.

Shipunov, A. 2003. The system of flowering plants: synthesis of classical and molecular approaches. *Zhurnal Obshchei Biologii*. 64: 501–509.

Shipunov, A. 2019. shipunov: Miscellaneous Functions from Alexey Shipunov. R package. URL: https://CRAN.R-project.org/package=shipunov (Accessed Mar 19 2020)

Shipunov, A. 2020. Ripeline and Rmanual speed up biological research and reporting. *arXiv* preprint arXiv:2002.01475.

Siebold, Fr. de & Zuccarini J.G. 1845. Florae Japonicae Familiae Naturales. Sec. 1. Abhandlungen der Mathematisch-Physikalischen Classe der Königlich Bayerischen Akademie der Wissenschaften. Munich. 4: 142.

Simmons, M.P, & Ochoterena, H. 2000. Gaps as Characters in Sequence-Based Phylogenetic Analyses. *Syst. Biol.* 49: 369–381.

Soh, W.K., von Sternburg, M., Hodkinson, T.R. & Parnell J.A. 2014. *Buxus sirindhorniana* sp. nov. (Buxaceae), a bicarpellate species from Thailand. *Nordic Journal of Botany*. 32: 452–458.

Soh, W.K. & Parnell J.A. 2018. Buxaceae. Flora of Thailand. 14: 9–13.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies, *Bioinformatics*. 30: 1312–1313.

Standley, P.C. 1936. Studies of American plants. VI. Field Museum of Natural History, Chicago.

Takahashi, M., Herendeen, P.S. & Xiao, X. 2017. Two early eudicot fossil flowers from the Kamikitaba assemblage (Coniacian, Late Cretaceous) in northeastern Japan. *Journal of Plant Research* 130: 809–826.

Takhtajan, A.L. 2009. Flowering Plants. Springer, New York.

Thouars, A.1804. *Histoire des Végétaux Recueillis sur les Îles de France, la Réunion (Bourbon) et Madagascar.* Huzard, Paris.

Torrez V. & Jørgensen, P.M. 2010. *Styloceras connatum* (Buxaceae), una nueva especie de Bolivia. *Novon*. 20: 363–366.

Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T. W., McNeill, J., Monro, A. M., Prado, J., Price, M. J. & Smith, G. F. (eds.) 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Koeltz Botanical Books, Glashütten

Ulloa, C.U., Acevedo-Rodríguez, P., Beck, S., Belgrano, M.J., Bernal, R., Berry, P.E., Brako, L., Celis, M., Davidse, G., Forzza, R.C. & Gradstein, S.R. 2017. An integrated assessment of the vascular plant species of the Americas. *Science*. 358(6370): 1614–1617.

van Laere K., Hermans D., Leus L. & van Huylenbroeck J. 2011. Genetic relationships in European and Asiatic *Buxus* species based on AFLP markers, genome sizes and chromosome numbers. *Plant Syst. Evol.* 293: 1–11.

van Tieghem, P. 1897. Sur les Buxacees. Ann. Sci. Nat. VIII. Bot. 5: 290-301.

von Balthazar, M. & Endress, P.K. 2002a. Development of inflorescences and flowers in Buxaceae and the problem of perianth interpretation. *Int. J. Plant Sci.* 163: 847–876.

von Balthazar, M. & Endress, P.K. 2002b. Reproductive structures and systematics of Buxaceae. *Bot. J. Linn. Soc.*. 140: 193–228.

von Balthazar, M., Endress, P.K. & Qiu, Y.L. 2000. Phylogenetic relationships in Buxaceae based on nuclear internal transcribed spacers and plastid *ndh*F sequences. *Int. J. Plant Sci.* 161: 785–792.

von Balthazar, M., Schatz, G.E. & Endress, P.K. 2003. Female flowers and inflorescences of Didymelaceae. *Plant Syst. Evol.* 237: 199–208.

Worberg, A., Quandt, D., Barniske, A.M., Löhne, C., Hilu, K.W., Borsch, T. 2007. Phylogeny of basal eudicots: insights from non-coding and rapidly evolving DNA. *Organisms Diversity & Evolution*. 7: 55–77.

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 (*trn*L, *rbc*L 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 (*trn*L, *rbc*L 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. *Didymeles 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.

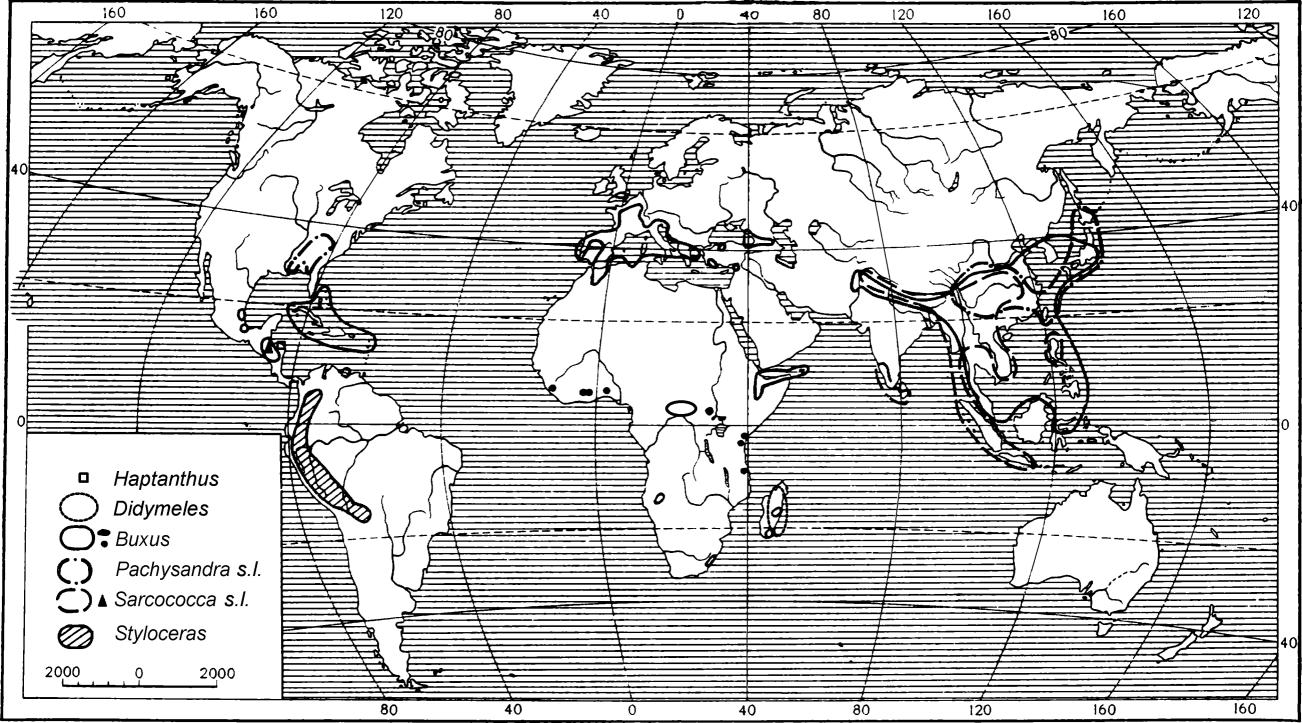
Support materials

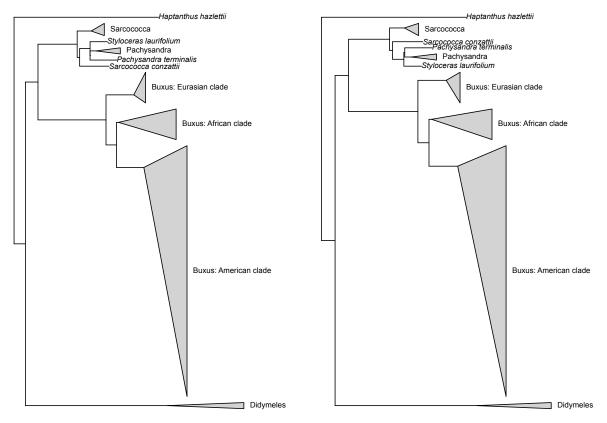
Support Table 1. Working classification of Buxaceae.

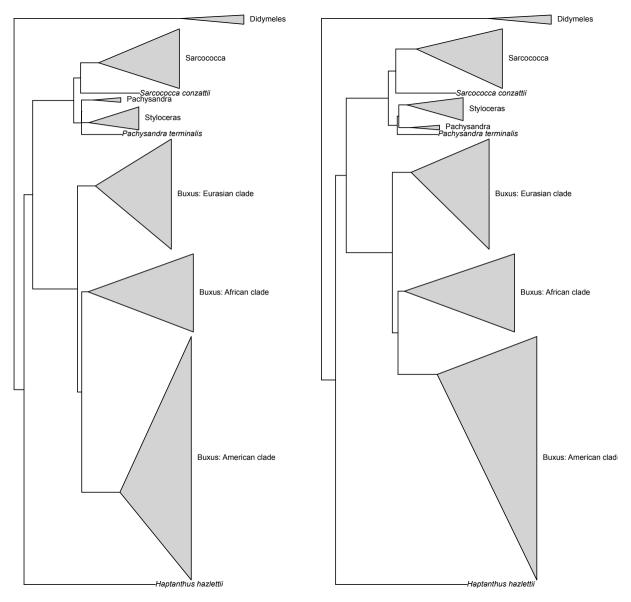
Support Table 2. Vouchers of Buxaceae samples.

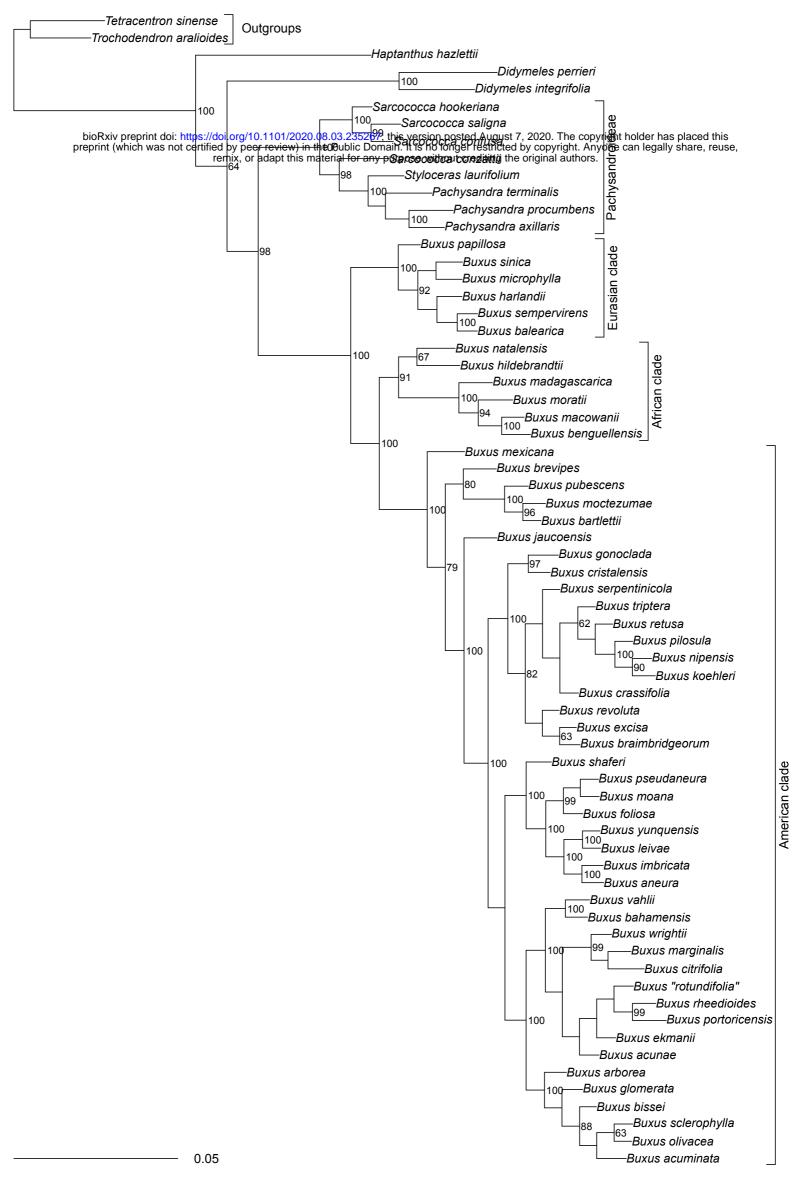
Support Table 3. GenBank accession numbers of Buxaceae samples sequenced for this study.

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

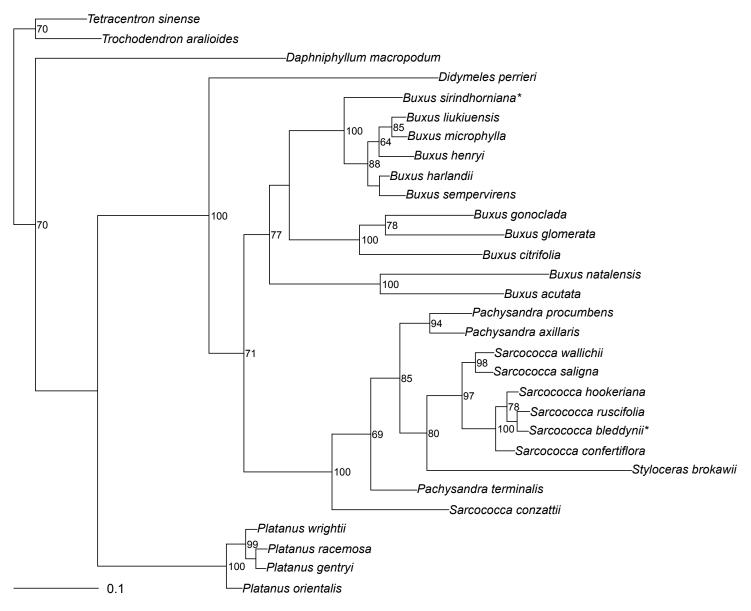




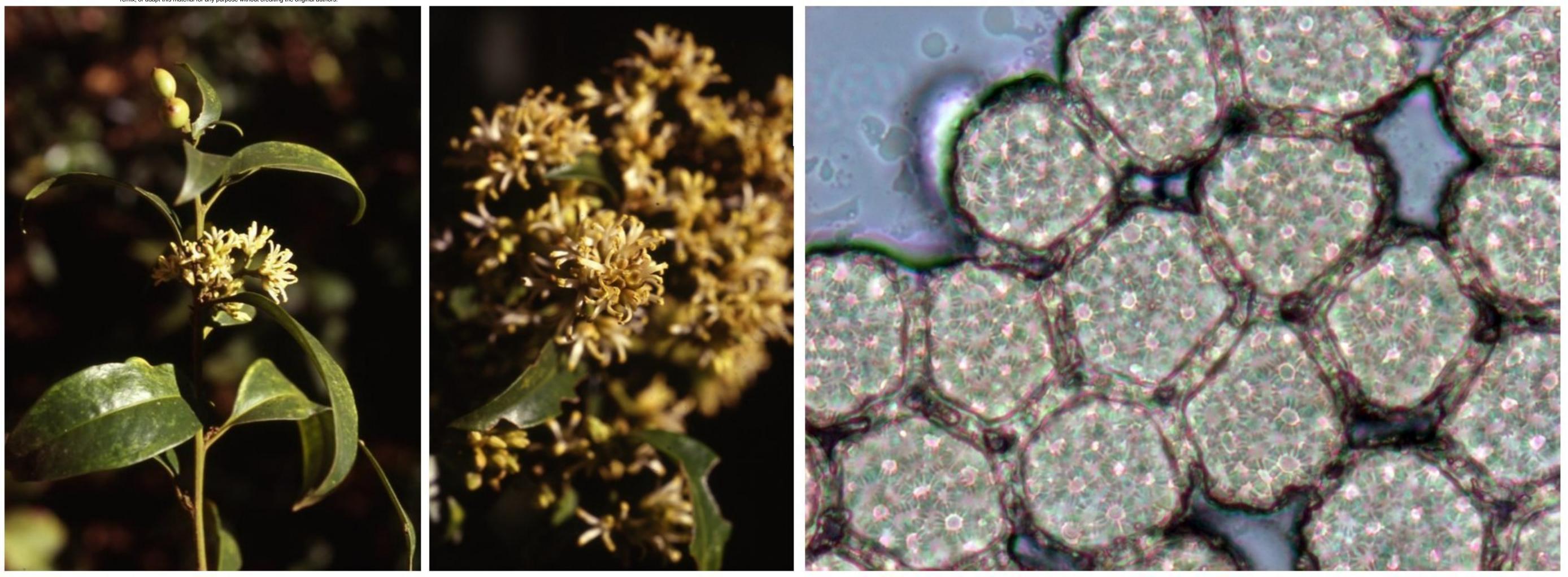












Buxaceae Dumort.

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.¹
 - 1(1). Didymeles integrifolia J.St.-Hil.
 - 2(2). Didymeles madagascariensis Willd., stat.m.²
 - 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.³
1(5). *Haptanthus hazlettii* Goldberg & C.Nelson

Subfamilia *Buxoideae* Beilschm.

Genus 3. Buxus L.

Subgenus Buxus⁴

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

¹Sometimes in Didymelaceae Leandri.

²Didymeles excelsa Baill.

³Sometimes in Haptanthaceae C.Nelson.

⁴Eubuxus Baill.

 $^{^5}Buxus\ longifolia\ Boiss.$

⁶Buxus holttumiana Hatus.

- 6(11). Buxus fortunei Carruth.
- 7(12). Buxus hainanensis Merr.
- 8(13). Buxus harlandii Hance⁷
- 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.⁸
- 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.Vidal⁹
- 27(32). Buxus rugulosa Hatus.
- 28(33). Buxus rupicola Ridl.
- 29(34). Buxus sempervirens L.¹⁰
- 30(35). Buxus sinica (Rehder & E.H.Wilson) M.Cheng¹¹
- 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 Friis¹²
- 35(40). Buxus benguellensis Gilg¹³
- 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 (Radel.-Sm.) Friis¹⁴

⁷Buxus cephalantha H.Lév. & Vaniot

⁸Buxus riparia (Makino) Makino

⁹Buxus philippinensis Rolfe, nom.nud.

¹⁰Buxus colchica Pojark.; Buxus hyrcana Pojark.

¹¹Buxus intermedia Kaneh.

¹²Notobuxus acuminata (Gilg.) Hutch.

¹³Notobuxus benguellensis (Gilg) E.Phillips; Buxus hirta (Hutch.) Mathou

¹⁴ Notobuxus cordata Radel.-Sm.

- 40(45). Buxus hildebrandtii Baill. 15
- 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. 16
- 45(50). Buxus macrocarpa Capuron
- 46(51). Buxus madagascarica Baill. 17
- 47(52). Buxus monticola G.E.Schatz & Lowry
- 48(53). Buxus moratii G.E.Schatz & Lowry
- 49(54). Buxus natalensis (Oliv.) Hutch. 18
- 50(55). Buxus nyasica Hutch.¹⁹
- 51(56). Buxus obtusifolia (Mildbr.) Hutch.²⁰
- 52(57). Buxus rabenantoandroi G.E.Schatz & Lowry²¹

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

- 53(58). Buxus acuminata (Griseb.) Müll.Arg.²²
- 54(59). Buxus acunae Borhidi & O.Muñiz
- 55(60). Buxus aneura Urb.
- 56(61). Buxus arborea Proctor
- 57(62). Buxus bahamensis Baker²³
- 58(63). Buxus baracoensis Borhidi & O.Muñiz²⁴
- 59(64). Buxus bartlettii Standl.
- 60(65). Buxus bissei Eg.Köhler
- 61(66). Buxus braimbridgeorum Eg.Köhler
- 62(67). Buxus brevipes (Müll.Arg.) Urb.²⁵
- 63(68). Buxus citrifolia (Willd.) Spreng.²⁶
- 64(69). Buxus crassifolia (Britton) Urb.²⁷
- 65(70). Buxus cristalensis Eg. Köhler & P.A.González
- 66(71). Buxus cubana (A.Rich.) Baill.²⁸
- 67(72). Buxus cyclophylla Floden & Shipunov, nom.nov.²⁹

¹⁵ Buxus calophylla Pax; Buxanthus hildebrantii Tiegh.; Buxus pedicellata (Tiegh.) Hutch.; Buxanthus pedicellatus Tiegh.

¹⁶Buxella macowanii Tiegh.; Notobuxus macowanii (Oliv.) E. Phillips

¹⁷Buxella madagascarica Tiegh.; Notobuxus madagascarica (Baill.) E. Phillips

¹⁸ Notobuxus natalensis Oliv.

¹⁹Notobuxus nyasica (Hutch.) E.Phillips

²⁰Notobuxus obtusifolia Mildbr.

²¹Buxus angustifolia G.E.Schatz & Lowry

²² Tricera acuminata Griseb.

²³ Tricera bahamensis (Baker) Britton

²⁴Buxus shaferi (Britton) Urb. p.p. sensu Eg.Köhler

²⁵ Tricera brevipes Britton

²⁶ Tricera citrifolia Willd.

²⁷ Tricera crassifolia Britton.

²⁸ Tricera cubana A.Rich.

 $^{^{29}\,}Tricera$ 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) Mathou³⁰
- 71(76). Buxus foliosa (Britton) Urb. 31
- 72(77). Buxus glomerata (Griseb.) Müll.Arg.³²
- 73(78). Buxus gonoclada (Griseb.) Müll.Arg.³³
- 74(79). Buxus heterophylla Urb.³⁴
- 75(80). Buxus historica Borhidi & O.Muñiz³⁵
- 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) Mathou³⁶
- 83(88). Buxus macrophylla (Britton) Fawc. & Rendle³⁷
- 84(89). Buxus marginalis (Britton) Urb.³⁸
- 85(90). Buxus mexicana Brandegee
- 86(91). Buxus moana Alain
- 87(92). Buxus moctezumae Eg.Köhler, R.Fernald & Zamudio
- 88(93). Buxus muelleriana Urb.³⁹
- 89(94). Buxus nipensis Eg. Köhler & P.A.González
- 90(95). Buxus obovata Urb.⁴⁰
- 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.⁴¹
- 99(104). Buxus revoluta (Britton) Mathou⁴²

³⁰ Tricera flaviramea Britton; Buxus gonoclada (Griseb.) Müll.Arg. p.p. sensu Eg.Köhler

³¹ Tricera foliosa Britton

³² Tricera glomerata Griseb.

³³ Tricera gonoclada Griseb.

³⁴Buxus gonoclada (Griseb.) Müll.Arg. p.p. sensu Eg.Köhler

³⁵Buxus retusa (Griseb.) Müll.Arg. p.p. sensu Eg.Köhler

³⁶ Tricera leonii Britton; Buxus wrightii subsp. leonii (Britton) Eg.Köhler

³⁷ Tricera macrophylla Britton

³⁸ Tricera marginalis Britton

³⁹ Tricera muelleriana Britton

⁴⁰Buxus shaferi (Britton) Urb. p.p. sensu Eg.Köhler

⁴¹ Tricera retusa Griseb.

⁴² 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.⁴³
- 104(109). Buxus subcolumnaris Müll.Arg.
- 105(110). Buxus triptera Eg.Köhler
- 106(111). Buxus vaccinioides (Britton) Urb.⁴⁴
- 107(112). Buxus vahlii Baill.
- 108(113). Buxus wrightii Müll.Arg.⁴⁵
- 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 Sweet⁴⁶
- 6(120). Sarcococca hookeriana Baill.⁴⁷
- 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 Stapf ex Sealy
- 12(126). Sarcococca ruscifolia Stapf⁴⁸
- 13(127). Sarcococca saligna (D.Don.) Müll.Arg.⁴⁹
- 14(128). Sarcococca wallichii Stapf
- 15(129). Sarcococca zeylanica Baill.⁵⁰
- 16(130). Sarcococca zollingeri Baill.

Genus 5. Sealya Floden & Shipunov, gen.nov.

1(131). Sealya conzattii (Standl). Floden & Shipunov, comb.nov. 51

 $^{^{43}\,} Tricera\ shaferi\ Britton$

⁴⁴ Tricera vaccinioides Britton

 $^{^{45}\,}Tricera\ microphylla$ Griseb.

⁴⁶Sarcococca nepalensis Royle; Sarcococca pruniformis Lindl. p.p.; Sarcococca tonkinensis Gagnep.; Sarcococca trinervia Wight; Sarcococca vagans Stapf

⁴⁷Sarcococca humilis Stapf

⁴⁸Sarcococca pauciflora C.Y.Wu

⁴⁹Sarcococca pruniformis Lindl. p.p.

 $^{^{50}}Sarcococca$ brevifolia (Muell. Arg.) Stapf ex Gamble

⁵¹Sarcococca 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.⁵²
- 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

⁵² Pachysandra stylosa Dunn

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

Buxus flaviramea	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)

Buxus rugulosa	B-721	Boufford 2884 (CAS-1011214)
Buxus sempervirens	B-003	No voucher, cultivated, photo in repository
Buxus sempervirens hyrcana	B-302	No voucher, photo in repository
Buxus shaferi	B-627	Brother Alain 4761 (US-2288281)
Buxus sinica	B-720	Yuan 1086 (CAS-846679)
Buxus sirindhorniana	B-500	van de Bult 664 (HUH)
Buxus sp. celebica	B-1817	PE-00044002
Buxus sp. Philippines	B-506	Reynoso 14113 (HUH)
Buxus stenophylla	B-1903	Hu 13026 (TI)
Buxus subcolumnaris	B-626	Howard 18878 (US-3001023)
Buxus vaccinioides	B-116	Shafer 4122 (NY)
Buxus vaccinioides	B-126	Shafer 4122 (NY)
Buxus vahlii	B-625	Little 21745 (US-2673754)
Buxus vahlii	B-807	Brother Alain 10722 (F-1618353)
Buxus wallichiana	B-615	Koelz 1831 (US-1605756)
Didymeles integrifolia	B-602	Schatz 2778 (US-3627925)
Didymeles madagascariensis	B-601	Rakotomazala 399 (US-3350972)
Didymeles perrieri	B-415	Razafimandribison 119 (MO-5617933)
Didymeles sp. Toamasina	B-417	Antilahimena 1529 (MO-6144923)
Didymeles sp. Toamasina	B-423	Antilahimena 2497a (MO-6177345)
Pachysandra axillaris	B-639	Hsien 1531 (US-1968483)
Pachysandra axillaris	B-703	Bonati s.n. (UC)
Pachysandra axillaris	B-714	Liu 15428 (CAS-943872)
Pachysandra procumbens	B-640	Kral 34 119 (US-2674034)
Pachysandra terminalis	B-005	No voucher, cultivated, photo in repository
Sarcococca balansae	B-303	No voucher, photo in repository
Sarcococca balansae	B-649	How 70661 (US-1675664)
Sarcococca balansae	B-811	How 70661 (F-779258)
Sarcococca bleddynii	floden3847	Floden s.n. (TENN)
Sarcococca confusa	B-713a	McClintock s.n. (CAS-582464)
Sarcococca conzattii	B-651	Panero 5574 (US-3661245)
Sarcococca conzattii	floden3834	Calzada 19727 (TENN)
Sarcococca conzattii	floden3835	Calzada 19727 (TENN)
Sarcococca conzattii	floden4073	Skutch 553 (TX)

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

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

Region	Sequenced fragment
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 its, rbcl, trnl

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 its, rbcl, trnl Mexico Guanxi its, rbcl Guanxi its, rbcl, trnl Cuba its, rbcl, trnl

rbcl

its, rbcl Okinawa Cape its, rbcl, trnl South Africa rbcl, trnl Madagascar its, rbcl, trnl rbcl, trnl Jamaica Madagascar its, rbcl Madagascar its, rbcl, trnl Madagascar its, rbcl Cuba rbcl, trnl Guangdong its, rbcl, trnl Mexico its, rbcl, trnl

Guanxi

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 rbcl, trnl Kenya 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, trnl
Madagascar its, rbcl
Cuba rbcl, trnl
Cuba trnl

Cuba its, rbcl, trnl
Cuba its, rbcl, trnl
Thailand its, rbcl, trnl
Cuba rbcl, trnl

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

rbcl Madagascar

its, rbcl, trnl Madagascar Madagascar its, rbcl, trnl

Madagascar rbcl Madagascar its Sichuan its, trnl Yunnan its Sichuan its, trnl its, trnl Tennessee **USA** its, rbcl Vietnam its, rbcl Hainan rbcl, trnl Hainan

Vietnam its, rbcl, trnl Cultivated its, rbcl, trnl Mexico rbcl, trnl Mexico rbcl, trnl Mexico its, trnl Guatemala its, trnl

rbcl

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 rbcl, trnl Bolivia Bolivia rbcl 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

its, rbcl, trnl

trnl

Peru

Peru

Peru its, rbcl, trnl

Ecuador rbcl
Peru rbcl
USA its
USA its

Sequence ID	Fragment	GenBank ID
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
B-207	its	MN537060
B-406	its	MN537061
B-412	its	MN537062
B-1901	its	MN537063
B-712b	its	MN537064
B-503	its	MN537065
B-112	its	MN537066
B-610	its	MN537067
B-706	its	MN537068
B-204	its	MN537069
B-617	its	MN537070
B-111	its	MN537071
B-636	its	MN537072
B-414	its	MN537073
B-104	its	MN537074
B-117	its	MN537075

B-614	its	MN537076
B-721	its	MN537077
B-003	its	MN537078
B-302	its	MN537079
B-627	its	MN537080
B-720	its	MN537081
B-500	its	MN537082
B-506	its	MN537083
B-1903	its	MN537084
B-626	its	MN537085
B-126	its	MN537086
B-615	its	MN537087
B-601	its	MN537088
B-415	its	MN537089
B-423	its	MN537090
B-639	its	MN537091
B-640	its	MN537092
B-005	its	MN537093
B-303	its	MN537094
floden3847	its	MN537095
B-713a	its	MN537096
floden3835	its	MN537097
floden4073	its	MN537098
B-648	its	MN537099
B-650	its	MN537100
B-004	its	MN537101
B-1812	its	MN537102
B-1811	its	MN537103
B-645	its	MN537104
B-644	its	MN537105
floden3971	its	MN537106
B-1801	its	MN537107
B-3005	its	MN537108
B-643	its	MN537109
B-605	its	MN537110
B-401	its	MN537111

B-403	its	MN537112
B-818	its	MN537113
B-301	its	MN537114
B-427	its	MN537115
B-001	its	MN537116
B-1816	its	MN537117
B-408	its	MN537118
B-1814	its	MN537119
B-413	its	MN537120
B-723	its	MN537121
B-411	its	MN537122
B-705	its	MN537123
B-703	its	MN537124
B-646	its	MN537125
B-701	its	MN537126
B-402	its	MN537127
B-400	its	MN537128
B-002	its	MN537129
B-611	its	MN537130
B-714	its	MN537131
B-712a	its	MN537132
B-603	its	MN537133
B-004	rbcl	MN579948
B-005	rbcl	MN579949
B-100	rbcl	MN579950
B-101	rbcl	MN579951
B-104	rbcl	MN579952
B-105	rbcl	MN579953
B-106	rbcl	MN579954
B-107	rbcl	MN579955
B-109	rbcl	MN579956
B-111	rbcl	MN579957
B-112	rbcl	MN579958
B-113	rbcl	MN579959
B-114	rbcl	MN579960
B-115	rbcl	MN579961

B-116	rbcl	MNEZOOGO
		MN579962
B-117	rbcl	MN579963
B-118	rbcl	MN579964
B-121	rbcl	MN579965
B-122	rbcl	MN579966
B-123	rbcl	MN579967
B-125	rbcl	MN579968
B-126	rbcl	MN579969
B-1601	rbcl	MN579970
B-1602	rbcl	MN579971
B-1603	rbcl	MN579972
B-1604	rbcl	MN579973
B-1801	rbcl	MN579974
B-1804	rbcl	MN579975
B-1805	rbcl	MN579976
B-1807	rbcl	MN579977
B-1808	rbcl	MN579978
B-1809	rbcl	MN579979
B-1810	rbcl	MN579980
B-1811	rbcl	MN579981
B-1812	rbcl	MN579982
B-1814	rbcl	MN579983
B-1815	rbcl	MN579984
B-1816	rbcl	MN579985
B-1817	rbcl	MN579986
B-1818	rbcl	MN579987
B-1819	rbcl	MN579988
B-1901	rbcl	MN579989
B-1902	rbcl	MN579990
B-1903	rbcl	MN579991
B-200	rbcl	MN579992
B-2001	rbcl	MN579993
B-201	rbcl	MN579994
B-202	rbcl	MN579995
B-204	rbcl	MN579996
B-205	rbcl	MN579997

B-206	rbcl	MN579998
B-207	rbcl	MN579999
B-2101	rbcl	MN580000
B-2151	rbcl	MN580001
B-2202	rbcl	MN580002
B-2801	rbcl	MN580003
B-3005	rbcl	MN580004
B-302	rbcl	MN580005
B-303	rbcl	MN580006
B-401	rbcl	MN580007
B-403	rbcl	MN580008
B-406	rbcl	MN580009
B-407	rbcl	MN580010
B-408	rbcl	MN580011
B-409	rbcl	MN580012
B-410	rbcl	MN580013
B-411	rbcl	MN580014
B-412	rbcl	MN580015
B-413	rbcl	MN580016
B-414	rbcl	MN580017
B-415	rbcl	MN580018
B-417	rbcl	MN580019
B-418	rbcl	MN580020
B-420	rbcl	MN580021
B-421	rbcl	MN580022
B-422	rbcl	MN580023
B-425	rbcl	MN580024
B-426	rbcl	MN580025
B-427	rbcl	MN580026
B-500	rbcl	MN580027
B-503	rbcl	MN580028
B-505	rbcl	MN580029
B-506	rbcl	MN580030
B-510	rbcl	MN580031
B-601	rbcl	MN580032
B-602	rbcl	MN580033

B-603	rbcl	MN580034
B-604	rbcl	MN580035
B-605	rbcl	MN580036
B-606	rbcl	MN580037
B-610	rbcl	MN580038
B-611	rbcl	MN580039
B-612	rbcl	MN580040
B-614	rbcl	MN580041
B-615	rbcl	MN580042
B-616	rbcl	MN580043
B-618	rbcl	MN580044
B-619	rbcl	MN580045
B-620	rbcl	MN580046
B-621	rbcl	MN580047
B-623	rbcl	MN580048
B-626	rbcl	MN580049
B-627	rbcl	MN580050
B-629	rbcl	MN580051
B-630	rbcl	MN580052
B-631	rbcl	MN580053
B-634	rbcl	MN580054
B-636	rbcl	MN580055
B-642	rbcl	MN580056
B-643	rbcl	MN580057
B-644	rbcl	MN580058
B-645	rbcl	MN580059
B-646	rbcl	MN580060
B-647	rbcl	MN580061
B-648	rbcl	MN580062
B-649	rbcl	MN580063
B-650	rbcl	MN580064
B-651	rbcl	MN580065
B-701	rbcl	MN580066
B-704	rbcl	MN580067
B-706	rbcl	MN580068
B-709	rbcl	MN580069

B-710	rbcl	MN580070
B-711	rbcl	MN580071
B-712a	rbcl	MN580072
B-712b	rbcl	MN580073
B-713a	rbcl	MN580074
B-713b	rbcl	MN580075
B-716	rbcl	MN580076
B-717	rbcl	MN580077
B-718	rbcl	MN580078
B-720	rbcl	MN580079
B-721	rbcl	MN580080
B-722	rbcl	MN580081
B-723	rbcl	MN580082
B-724	rbcl	MN580083
B-802	rbcl	MN580084
B-803	rbcl	MN580085
B-807	rbcl	MN580086
B-808	rbcl	MN580087
B-809	rbcl	MN580088
B-810	rbcl	MN580089
B-811	rbcl	MN580090
B-817	rbcl	MN580091
floden3834	rbcl	MN580092
floden3847	rbcl	MN580093
B-420	trnl	MN549158
B-118	trnl	MN549159
B-101	trnl	MN549160
B-722	trnl	MN549161
B-635	trnl	MN549162
B-716	trnl	MN549163
B-202	trnl	MN549164
B-1819	trnl	MN549165
B-103	trnl	MN549166
B-122	trnl	MN549167
B-426	trnl	MN549168
B-425	trnl	MN549169

B-1807	trnl	MN549170
B-1808	trnl	MN549171
B-205	trnl	MN549172
B-623	trnl	MN549173
B-621	trnl	MN549174
B-200	trnl	MN549175
B-422	trnl	MN549176
B-100	trnl	MN549177
B-114	trnl	MN549178
B-107	trnl	MN549179
B-106	trnl	MN549180
B-631	trnl	MN549181
B-632	trnl	MN549182
B-717	trnl	MN549183
B-718	trnl	MN549184
B-119	trnl	MN549185
B-409	trnl	MN549186
B-710	trnl	MN549187
B-407	trnl	MN549188
B-123	trnl	MN549189
B-713b	trnl	MN549190
B-1814	trnl	MN549191
B-121	trnl	MN549192
B-207	trnl	MN549193
B-406	trnl	MN549194
B-630	trnl	MN549195
B-413	trnl	MN549196
B-629	trnl	MN549197
B-1901	trnl	MN549198
B-712b	trnl	MN549199
B-704	trnl	MN549200
B-724	trnl	MN549201
B-503	trnl	MN549202
B-112	trnl	MN549203
B-418	trnl	MN549204
B-616	trnl	MN549205

B-706	trnl	MN549206
B-110	trnl	MN549207
B-810	trnl	MN549208
B-617	trnl	MN549209
B-111	trnl	MN549210
B-816	trnl	MN549211
B-636	trnl	MN549212
B-802	trnl	MN549213
B-105	trnl	MN549214
B-104	trnl	MN549215
B-117	trnl	MN549216
B-614	trnl	MN549217
B-115	trnl	MN549218
B-721	trnl	MN549219
B-302	trnl	MN549220
B-627	trnl	MN549221
B-720	trnl	MN549222
B-500	trnl	MN549223
B-1903	trnl	MN549224
B-626	trnl	MN549225
B-126	trnl	MN549226
B-625	trnl	MN549227
B-615	trnl	MN549228
B-601	trnl	MN549229
B-415	trnl	MN549230
B-639	trnl	MN549231
B-640	trnl	MN549232
B-649	trnl	MN549233
floden3847	trnl	MN549234
B-713a	trnl	MN549235
B-651	trnl	MN549236
B-650	trnl	MN549237
B-004	trnl	MN549238
B-1812	trnl	MN549239
B-1811	trnl	MN549240
B-2001	trnl	MN549241

B-645	trnl	MN549242
B-644	trnl	MN549243
floden3971	trnl	MN549244
B-1801	trnl	MN549245
B-643	trnl	MN549246
B-647	trnl	MN549247
B-2101	trnl	MN549248
B-1604	trnl	MN549249
B-2801	trnl	MN549250
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
B-612	trnl	MN549260
B-723	trnl	MN549261
B-411	trnl	MN549262
B-610	trnl	MN549263
B-705	trnl	MN549264
B-502	trnl	MN549265
B-807	trnl	MN549266
B-714	trnl	MN549267
floden3834	trnl	MN549268
B-646	trnl	MN549269
B-701	trnl	MN549270
B-818	trnl	MN549271
B-603	trnl	MN549272
floden3835	trnl	MN549273
B-712a	trnl	MN549274
B-815	trnl	MN549275
floden4073	trnl	MN549276
B-1603	trnl	MN549277

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

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 conzattii	petd
LN877592	Sarcococca confusa	petd
AM396541	Pachysandra terminalis	petd
LN877605	Haptanthus hazlettii	petd
AM396540	Didymeles integrifolia	petd
LN877548	Buxus yunquensis	petd

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

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	Didymeles 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