Review

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Genomic insights into the genus *Buxus* and boxwood blight

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Abstract
The genus *Buxus*, encompassing approximately 70 species, is highly valued for its ornamental appeal and is widely used in landscaping. These evergreen shrubs are predominantly found in Europe, Asia, Africa, the Caribbean, and parts of India and the Himalayas, but are absent in temperate North America and Australia. Nowadays, the genus faces significant threats from boxwood blight, a disease caused by *Calonectria pseudonaviculata* and *Calonectria henricotiae*, which leads to rapid leaf blight and defoliation, potentially reducing genetic diversity within *Buxus* species. Recent genomic studies have advanced our understanding of *Buxus* species and the pathogens affecting them. This review article summarizes the key findings from recent decades genomic studies on genus *Buxus* and boxwood blight, emphasizing the importance of genetic research in managing and preserving these valuable ornamental plants.

Keywords
Buxus, Boxwood, Boxwood blight, Genomic studies.

Introduction
The *Buxus* genus, commonly known as boxwood or box tree, is the largest genus of the Buxaceae family and encompasses around 70 species. These evergreen shrubs are highly valued for their ornamental appeal and are used in various forms such as individual plants, hedges, parterres, clusters, container plants, and topiaries. Moreover, *Buxus* species are utilized in traditional medicine for their anticholinesterase, anti-HIV, cytotoxic, and immunosuppressive properties (Amtaghri, & Mohamed Eddouks, 2024). Boxwoods are found in five major geographical areas around the world: (1) Europe, the Mediterranean Basin, the Middle East, (2) China, Japan, Korea, Malaysia, the Philippines, (3) Africa, (4) the Caribbean Islands, Mexico, South America,

and (5) India, the northwest Himalayas, the Eurasian region. Indigenous species of *Buxus* are not found in temperate North America and Australia. Most species thrive in tropical and subtropical climates, although some species in Europe and Asia are resistant to frost (von Balthazar *et al.*, 2000).

Based on visible characteristics, *Buxus colchica* from the western Caucasus and *Buxus hyrcana* from Iran are often considered as synonyms of *B. sempervirens* (Med-Checklist, 2007; Botanica Sistematica, 2007). The evergreen shrub *B. sempervirens* can grow between 1 to 9 meters tall, with a trunk diameter of up to 20 cm. Its leaves are green to yellow-green, oval-shaped, and measure 1.5-3 cm in length and 0.5-1.3 cm in width. referred to as the common box, European

The most well-known species of the *Buxus* genus is *Buxus sempervirens*, commonly box, or boxwood. This species is native to western and southern Europe, northwest Africa, and southwest Asia, ranging from southern England down to northern Morocco and across the northern Mediterranean to Turkey (Rushforth *et al.*, 1999; World Flora Online, n.d., 2025) (Fig. 1).
The flowers are hermaphroditic, and the fruits are three-lobed capsules containing 3 to 6 seeds (Rushforth *et al.*, 1999) (Fig. 2). Based on the evaluation of archaeological artifacts from Holocene sites in Spain, it is reported that *B. sempervirens* was success fully used by prehistoric societies to manufacture various objects (e.g., sickle handles, digging sticks, wedges, adze handles, needles, combs) (Pique *et al.*, 2018).

Fig. 1. Distribution map of *Buxus sempervirens* (European box). The native continuous range is shown in green, while native isolated populations are marked with "x" (Caudullo *et al.*, 2017).

Fig. 2. *Buxus sempervirens* (European box) photographed in its natural habitat in the Georgia (Photo: B. Berdzenishvili).

Nowadays, boxwood blight is recognized as a disease that widely affects decorative and native boxwood plants in the Buxaceae family. Initially identified in the 1990s in England, it has since spread across Europe, Asia, New Zealand, and North America. The disease is caused by two distinct but related species of the *Calonectria* fungi: *Calonectria pseudonaviculata* and *Calonectria henricotiae* (LeBlanc *et al.*, 2018; Daughtrey *et al.*, 2019). It should be mentioned that boxwood blight also affects two other plants in the Buxaceae family: sweet box (*Sarcococca* spp.) and pachysandra (*Pachysandra* spp.). The most characteristic features of boxwood blight are enormously rapid leaf blighting and defoliation under optimal environmental conditions (Daughtrey *et al.*, 2019). The widespread existence of boxwood blight disease is quite dangerous because it can be reason of decrease in the genetic diversity of *Buxus* species in the future.

From a botanical perspective, boxwood has been well described, and the morpho-botanical characteristics of different species have been well documented. Meanwhile, in the literature, several interesting genetic studies on the genus can be found but amount of such studies is less than any other subject papers regarding boxwoods. Scientific articles on *Buxus* genome research can be broadly divided into two main categories: nuclear genomics and plastome genomics. A distinct category includes literature focused on genetic methods for detecting and identifying boxwood blight.

Recent advances in the understanding of plant genetics and genomics have led to significant progress in the field. These studies provide valuable insights into the potential applications of genomic tools and technologies for crop improvement and sustainable agriculture, while also addressing fundame-

ntal questions regarding the evolution and function of plant genes and genomes. The purpose of this review paper is to provide an overview of scientific research on genomic studies of *Buxus* and boxwood blight in last decades.

Nuclear genome researches of genus *Buxus*

A significant part of genetic studies and therefore research papers on *Buxus* species involves studies utilizing various molecular markers, such as AFLP (Amplified Fragment Length Polymorphism), ITS (Internal Transcribed Spacer), SSR (Genic Simple Sequence Repeat) and ISSR (Inter-Simple Sequence Repeat), among others.

A notable study on *Buxus* nuclear genomes by Laere *et al.,* (2011) reported the existence of two distinct clusters among European and Asian *Buxus* species. This conclusion was based on analyses of AFLP, genome size, and chromosome counts. One cluster includes the European species *B. sempervirens*, *B. balearica*, and *B. colchica*. The second cluster comprises Asiatic *Buxus* species, namely *B. microphylla*, *B. harlandii*, *B. hyrcana*, *B. myrica*, *B. henryi*, *B. bodinieri*, and *B. wallichiana*. In the first cluster, all species were diploids (2n=38) except for *B. sempervirens*, which was triploid. The genome size in this cluster ranged between 1.38 and 1.69 pg 2C(-1). The ploidy levels in the second cluster were more varied. Specifically, *B. harlandii*, *B. hyrcana*, and nine *B. microphylla* cultivars were tetraploid (2n = 4x = 56) with a genome size greater than 2.5 pg 2C(-1). Some *B. microphylla* cultivars were triploid (2n = 3x = 42) with a genome size greater than 2.5pg 2C(-1), while Asiatic *B. henryi*, *B. bodinieri*, and some *B. microphylla* cultivars were diploid with a genome size of approximately 1.5 pg 2C(-1).

By incorporating the rDNA ITS sequences of nine *Buxus* species from GenBank and using the ITS sequence of *Pachysandra terminalis* as a reference, Wang *et al.*, (2012) conducted an alignment analysis for the ITS sequences of 18 *Buxus* species. The analysis revealed that nucleotide substitution in the ITS sequences of *Buxus* plants predominantly involves transitions rather than transversions. In the ITS-1 region, the number of transitioning bases is nearly equal to that of transversing bases, while in the ITS-2 region, transitions outnumber transversions. A similar pattern is observed in the 5.8S region. This study suggests that the Chinese pearl boxwood (*B. sinica* var. *parvifolia*) should be considered a sister species rather than a variant of *B. sinica*, based on relative detection rates and phylogenetic analysis. Additionally, there is a close genetic relationship between *B. sinica* var. *parvifolia* and *B. henryi*.

Thammina *et al.* (2014), aiming to enhance genetic diversity analysis, taxon identification, and facilitate breeding in *Buxus* species, developed the first 23 genic-SSR markers for this purpose. In this study a cDNA library was created from mRNA extracted from the leaves of *Buxus sempervirens* 'Vardar Valley' and sequenced using the Illumina MiSeq platform. Analysis of approximately 11.9 million base pairs revealed 845 genic-SSRs, including various repeat types: 469 dinucleotides, 360 trinucleotides, seven tetranucleotides, one pentanucleotide, and eight hexanucleotides. From these, 71 trinucleotide repeat-containing genic-SSRs

were selected, and primer pairs were developed to amplify loci across 18 different boxwood accessions. Out of these, 23 primer pairs successfully amplified polymorphic loci, with two to 10 alleles identified at each locus. It was concluded that the developed markers could be successfully used for characterizing the genetic diversity and relatedness of boxwood germplasm.

Another notable study conducted by Thammina *et al.*, (2017) used flow cytometry and genic-SSR markers to assess 275 accessions of *Buxus* from the National Boxwood Collection at the U.S. National Arboretum. The study focused on exploring genetic diversity and relatedness among the studied plant accessions. The results revealed the presence of two major clusters, each further subdivided into four subclusters. These clusters included *B. balearica* Lam., *B. bodinieri* Le´vl., *B. harlandii* Hance, *B. microphylla* Siebold et Zuccarini, *B. sempervirens* L., *B. sinica* (Rehd. et Wils.) M. Cheng, and their putative interspecific hybrids. The accessions were grouped based on cultivar, provenance, or species. Within each group, clustering typically reflected known breeding pedigrees, and the robustness of these clusters was supported by bootstrap analysis.

Randomly Amplified Polymorphic DNA (RAPD) and ISSR molecular markers were used in a study involving Iranian *Buxus hyrcana*, which examined 15 healthy and 15 infected trees from each of two populations. The analysis indicated that genetic diversity parameters were generally higher in healthy trees compared to infected ones (Shanjani *et al.*, 2018).

One more study into *Buxus hyrcana* genetic diversity and differentiation involved 97 accessions from seven geographic regions of Hyrcanian forests in Iran, utilizing 10 ISSR primers (Esmaeilnezhad *et al.*, 2020). This study revealed that inter-population genetic diversity was higher than intra-population genetic diversity, with geographic and environmental factors playing a significant role in shaping regional gene flow and influencing the genetic structure of *B. hyrcana* populations.

Additionally, RAPD molecular marker analysis of Northern Iranian *Buxus hyrcana* showed relatively low genetic diversity within populations from four regions, although the highest diversity was observed within each population. This finding is significant as it contributes to identifying inherently or environmentally resistant *Buxus hyrcana* species against fire blight disease (Mana *et al.*, 2021).

By analyzing of the high-quality chromosome-level genome of *Buxus austro-yunnanensis* (Buxales) Wang *et al.*, (2022) show that *Buxales* and *Trochodendrales* are closely related sister groups and that both are sisters to the core eudicots. The study found that incomplete lineage sorting and hybridization contribute to 34.33% of the phylogenetic discordance between lineages. *Buxus austro-yunnanensis* has undergone a single whole-genome duplication event, with independent polyploidizations occurring in five eudicot lineages. Authors reconstructed the ancestral eudicot karyotype (AEK) using representative genomes and created nearly gapless karyotype projections for each eudicot species.

The study by Chanderbali *et al.*, (2022) is particularly intriguing as it focuses on resolving the genome history of *Buxus* and *Tetracentron* within the context of eudicots. They found that both *Buxus* and *Tetracentron* have undergone independent whole-genome duplications, which helped elucidate relationships among early-diverging eudicots and their respective genome structures. Using the RACCROCHE reconstruction pipeline, the study demonstrated that the genome structure remained relatively stable during early eudicot diversification. This finding contradicts hypotheses suggesting that the gamma duplication arose from inter-lineage hybridization between ancestral eudicot lineages. Instead, the study proposed that the gamma duplication event involved only the stem lineage core eudicot ancestors. This research sheds light on the evolutionary dynamics of eudicot genomes and provides insights into their complex history.

A significant work was also made on the genome assembly of *B. sempervirens* by Christenhusz *et al.* (2024). The genome material used in this study were taken from a plant cultivated at the Royal Botanic Gardens, Kew, of known wild source (Box Hill, Surrey). The genome was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 22.84 Gb from 1.64 million reads. The total length of the genome sequence was 676.70 megabases. 99.56% of the assembly was scaffolded into 14 chromosomal pseudomolecules while the plastid genome assembly was 150.93 kilobases in length. Besides, 8 mitochondrial sequences were also assembled.

With the rise of genome-based studies, more *Buxus* genomic sequences are becoming available in specialized databases. The largest publicly available biological database, NCBI provides access to the whole-genome sequencing and annotation results of *Buxus sempervirens*' chromosomal, chloroplast, and mitochondrial genomes, contributed by the Wellcome Sanger Tree of Life Programme (Table 1). Additionally, NCBI provides a wide range of specific DNA regions from various regions of *Buxus* genome (i. e. nucleotide sequences of *trn*T-*trn*L intergenic spacer, *trn*L gene and *trn*L-*trn*F intergenic spacer, GenBank: AY145357.1; maturase K (*mat*K) gene, GenBank: MK926102.1; large subunit ribosomal RNA gene, GenBank: DQ629-363.1; tRNA-Leu gene (partial) and *trn*L-F IGS (partial), GenBank: LN877669.1; *nad*5 gene, GenBank: DQ406879.1; RPB1 gene, GenBank: DQ228258.1; *ndh*F gene, GenBank: AF241600.1, etc.).

It would be underlined that genome-based researches, especially whole genome sequencing, is crucial as it enables a detailed examination of an organism's genomic structure and the molecular basis of its characteristic traits, with implications for both fundamental and applied science.

Plastome genomics of genus *Buxus*

Plastome or chloroplast genome is an excellent resource for taxa identification, plant phylogeny and evolutionary history studies. Unlike standard genomic barcodes, which typically consist of short DNA sequ-

Table 1. Whole-genome sequences of *Buxus sempervirens* (chromosomal, chloroplast, and mitochondrial) available from NCBI (see References).

Definition	GenBank accession
Buxus sempervirens whole genome shotgun sequencing project	CANNZT000000000.2 CANNZS000000000.3
Buxus sempervirens genome assembly, chromosome: 14	OX387195.1
Buxus sempervirens genome assembly, chromosome: 13	OX387194.1
Buxus sempervirens genome assembly, chromosome: 12	OX387193.1
Buxus sempervirens genome assembly, chromosome: 11	OX387192.1
Buxus sempervirens genome assembly, chromosome: 10	OX387191.1
Buxus sempervirens genome assembly, chromosome: 9	OX387190.1
Buxus sempervirens genome assembly, chromosome: 8	OX387189.1
Buxus sempervirens genome assembly, chromosome: 7	OX387188.1
Buxus sempervirens genome assembly, chromosome: 6	OX387187.1
Buxus sempervirens genome assembly, chromosome: 5	OX387186.1
Buxus sempervirens genome assembly, chromosome: 4	OX387185.1
Buxus sempervirens genome assembly, chromosome: 3	OX387184.1
Buxus sempervirens genome assembly, chromosome: 2	OX387183.1
Buxus sempervirens genome assembly, chromosome: 1	OX387182.1
Buxus sempervirens genome assembly, organelle: mitochondrion	OZ124165.1, OZ124164.1 OZ124163.1, OZ124162.1 OZ124161.1, OZ124160.1 OZ124159.1, OZ124158.1
Buxus sempervirens genome assembly, organelle: plastid: chloroplast	OZ124166.1

ences (400-800 bp) with considerable genetic variation, barcoding through chloroplast genome sequencing can differentiate closely related species. Plastids usually are inherited uniparentally, do not recombine and existing like one single genome locus in the cell, what makes them very useful for the phylogenetically studies (Ahmad *et al.*, 2023; Fu *et al.*, 2021; Savolainen *et al.*, 2005). In recent years, the availability of Next-generation sequencing (NGS) and improvements in genome analysis approaches have made extensive plastome datasets for various plant species accessible in GenBank, including some genomes of *Buxus* species (Hansen *et al.*, 2007; He *et al.*, 2021; Yin *et al.*, 2024; Pipia *et al.*, 2023; Pipia *et al.*, 2024).

Back in 2007, Hansen *et al.*, published a scientific article presenting the first complete chloroplast genome sequence of the Asian species *Buxus microphylla* (GenBank Accession No. NC_009599), along with the complete chloroplast genomes of three other plants: *C. spicatus* (NC_00-9598), *D. elephantipes* (NC_00-9601), and *I. oligandrum* (NC_009600). Specifically, in the chloroplast genome of *Buxus microphylla*, more than 130 protein-coding genes were identified, and the whole plastome was defined to be 159,010 bp in length. All four

genomes are available at NCBI under afford mentioned GenBank number. Genome assessment showed that these shown that these chloroplast genomes are very similar in size, gene content, and gene order to the ancestral angiosperm genome represented by Amborella, Nuphar, and Nymphaea (Hansen *et al.*, 2007).

In the study provided by Gutiérrez *et al.,* (2013) three new species of *Buxus*, endemic to the serpentine areas of Sierra de Nipe and Sierra del Cristal in northeastern Cuba, were described. The species were characterized through morphological descriptions, including pollen and leaf anatomy, and molecular descriptions based on plastid *trn*K-*mat*K and *trn*L-*trn*F regions. Substitutions within these sequences were evaluated to identify molecular characters that could complement the morphological diagnosis. The newly described species of *Buxus* serve as an example to discuss the prospects and challenges of using DNA characters for species diagnosis. Additionally, an assessment of the distribution, habitat, ecology, and conservation status of these three newly recognized endemic species was provided (Gutiérrez *et al.,* 2013).

Seventeen haplotypes were identified by sequencing 340 individuals from 65 populations of *B. sempervirens* and *Buxus balearica* across the western Palearctic region. Most populations exhibited fixation for a single haplotype, reflecting high population differentiation, low within-population variability, and an absence of phylogeographic structure in cpDNA diversity. All 17 haplotypes (100%) were found south of 43°N, where *Buxus* populations are fragmented and have undergone significant reductions over the past few millennia. In contrast, a single haplotype dominates central and western Europe, where populations are more continuous and experienced rapid expansion during the Holocene. Mutational differences between haplotypes suggest distinct evolutionary trajectories among Mediterranean Peninsulas, with notable divergence between the western-central regions (Iberian and Italian) and central-eastern areas (Balkan and Anatolian) (Di Domenico *et al.*, 2013).

Yao *et al.*, (2021) characterized the plastome of Chinese *Buxus megistophylla* using Illumina paired-end sequencing and conducted phylogenetic analyses. The research revealed that the chloroplast genome was 157,611 bp in length, consisting of an 85,930 bp large single-copy region (LSC), an 18,319 bp small single-copy region (SSC), and two 26,681 bp long inverted repeat (IR) regions. The plastome contained 124 genes, including 89 coding genes, 31 tRNA genes, and 4 rRNA genes. The phylogenetic study conducted in the same research indicated that *Pachysandra terminalis* (Japanese spurge) and *Buxus microphylla* are closely related and clustered under the same node. In contrast, *Buxus megistophylla* appeared relatively distant from them. These taxa formed a clade and were somewhat closer to each other compared to species from other families.

In the study by Yin *et al.* (2024), the chloroplast genome of *B. sinica* var. *parvifolia* was sequenced for the first time using the

Illumina HiSeq 2500-PE150 platform (GenBank: OQ236088.1). The genome was assembled and annotated with GetOrganelle software and Geneious v2022.2.2. It was detected that the chloroplast genome of *B. sinica* var. *parvifolia* is 158,995 bp in length and comprises one LSC region, one SSC region, and two IR regions. In the sequenced plastome, 132 genes were annotated, including 87 protein-coding genes, 37 tRNA genes, and 8 rRNA genes. Phylogenetic analysis conducted in this study revealed a sister relationship between *B. sinica* var. *parvifolia* and *B. microphylla*.

Genome studies of boxwood blight causing pathogens

The are identified two main pathogens causing boxwood blight: *Calonectria pseudonaviculata* and *Calonectria henricotiae*. *Calonectria pseudonaviculata* is widely distributed Worldwide including North America, western Asia, New Zealand and Europe, while *Calonectria henricotiae* has only been observed in Europe so far (Castroagudín *et al.*, 2020; Yang *et al.*, 2022; Kong *et al.*, 2024). The detection of the affected plants can be made by visual symptomatic characteristics, such as leaf spots or blotches (brown to black specks), rapid defoliation, and stem lesions (dark brown to black) (Castroagudín *et al.*, 2020). The precise identification of boxwood blight pathogens in both asymptomatic and symptomatic plants is enabled by molecular diagnostic techniques (i.e. RT-PCR, LAMP assays, whole-genome sequencing).

Malapi-Wight *et al.*, (2016) aimed to find target genome regions appropriate for the diagnosis of *Calonectria henricotiae* and *C. pseudonaviculata*. Based on comparative genomics datasets (unique regions, polymorphisms, presence/absence of regions across datasets), it was demonstrated that the LAMP (loop-mediated isothermal amplification) diagnostic assay could be easily applied for diagnosing fungi and other newly emergent plant pathogens.

In order to prevent the rapid and large-scale spread of the disease, it is necessary to detect it quickly. In the scientific literature some very interesting papers can be found aimed to effective detection of boxwood blight disease. For instance, the first published diagnostic assays developed for boxwood blight were based on real-time PCR detection of the multiple-copy rDNA internal transcribed spacer (ITS) and the single-copy β-tubulin 2 (*TUB2*) gene and PCR-RFLP (restriction fragment length polymorphism) assay (Gehesquière *et al.*, 2016; LeBlanc *et al.*, 2018). Some authors are reported that LAMP diagnostic assay is also effective method for pathogen detection in plants.

The geographically disparate populations of boxwood blight causing *C. pseudonaviculata* (n = 19) and *C. henricotiae* (n = 7) from the U.S., Europe, Asia, and New Zealand were analyzed for polymorphism in CYP51 paralogs. CYP51 (14α-demethylase) is a fungal-specific enzyme essential for producing ergosterol, a key sterol in fungal cell membranes. Azoles inhibit ergosterol synthesis by binding to this enzyme's active

site, leading to the weakening and disruption of the fungal cell membrane. In all studied *C. pseudonaviculata*, the presence of a CYP51A pseudogene and the absence of a functional CYP51A paralog were shown for the first time in fungi. This observation can be important for the development of resistance to antifungal chemicals (Stravoravdis *et al.*, 2019).

A total of 1,608 single-nucleotide polymorphisms (SNPs) were identified through whole-genome sequencing in 67 C*. pseudonaviculata* isolates from four continents, and 1,017 SNPs were found in 13 *C. henricotiae* isolates from Europe. Interspecific genetic differentiation and the absence of shared polymorphisms were observed, indicating a lack of gene flow between the sister species (LeBlanc *et al.*, 2020).

The article by Guo and co-authors (Guo *et al.*, 2020) addresses the development of experimental tools for rapid and efficient disease detection. In particular, the authors examined pairs of primers for calmodulin, histone H3, internal transcribed spacer, and β-tubulin conserved regions to differentiate between the two disease-causing species: *C. pseudonaviculata* and *C. henricotiae*. In the course of this work, three primer pairs based on the histone H3 region were derived, which can be used to detect both *C. pseudonaviculata* and *C. henricotiae*. It is important that the mentioned primers can be used in either multiplexed conventional PCR or SYBR-based real-time PCR.

A total of 19,750 gene families were identified in the 24 genomes, of which 422 were found to be rapidly evolving. Among the six *Calonectria* species, high levels of rapid contraction of pathogenesis-related gene families were experienced only by *C. henricotiae* and *C. pseudonaviculata* (89% and 78%, respectively). In contrast, rapid expansion of pathogenesis-related gene families was observed in the saprobic species *Calonectria multiphialidica* and *C. naviculata*, two of the nearest known relatives of *C. henricotiae* and *C. pseudonaviculata*. Novel insight into gene family evolution within *C. henricotiae* and *C. pseudonaviculata* provided by this study, suggesting that gene family contraction may have contributed to the limited host-range expansion of these pathogens within the plant family Buxaceae (Rogers *et al.*, 2022).

Yang *et al.*, (2022) applied metagenomic sequencing with the Oxford Nanopore Technologies MinION to the detection of the fungus *Calonectria pseudonaviculata*. It has been shown that by using appropriate DNA extraction techniques, bioinformatics tools, genome databases and metagenomic sequencing with the ONT MinION can be used to easily distinguish the boxwood blight pathogens *Calonectria pseudonaviculata* and *Calonectria henricotiae* from each other as well as from other fungal species.

Conclusions

The *Buxus* genus is globally valued for its ornamental use and is commonly utilized in landscaping and decorative gardening. However, boxwood blight, caused by Calonectria fungi, poses a significant threat to these plants. Research on *Buxus* species

and their pathogens has made notable progress, with molecular markers and genome sequencing providing insights into their genetic structure and diversity, evolution, potential for breeding, and susceptibility to boxwood diseases. Continued genomic research, especially focusing on *Buxus* genomes, pathogen detection, resistance, and the genetic basis of boxwood traits, will be key to preserving the biodiversity and resilience of *Buxus* species.

Conflict of interest

The authors declare that they do not have any conflicts of interest.

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