

The Genus *Buxus* (*Buxaceae*)

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RESUMEN

Se discute el papel de los patrones de nerviación del pecíolo y de la hoja, la evolución de la apertura del grano de polen y los caracteres de la exina en *Buxus* en relación con su disjunta distribución global y la clasificación del género. Los primeros *taxones* están representados en el este de África mientras tres ramas independientes, con grupos parafiléticos claramente diferenciables se encuentran en América, este de Asia y África meridional. La notable evolución paralela de la apertura del polen y los caracteres de la exina en los diferentes continentes sirve de base a esta agrupación y se interpreta como expresión de un ancestro básico común. Por el contrario, los patrones de nerviación de la hoja reflejan claramente la diferenciación evolutiva entre los grupos continentales y dentro de ellos. Cuba representa el centro de evolución del Nuevo Mundo con el mayor número de especies y la mayor diversidad morfológica y filogenética en la que solo unos pocos grupos filéticos migraron hacia otras islas de Las Antillas y América Central.

Palabras clave: *Buxus*, taxonomía, polen, evolución.

ABSTRACT

Patterns of leaf petiole and axis vascularization, of pollen grain aperture evolution and exine characters as well as characters of leaf venation of *Buxus* are discussed in relation to the disjunct global distribution and classification of the genus. The most original taxa are represented in East Africa, while three independent branches with clearly separated phyletic groups occur in America, in East Asia and in South Africa. The remarkable parallel evolution of aperture and exine characters on the different continents support this grouping and is interpreted as expression of a common ancestral genetic basis. In contrast the patterns of leaf venation clearly reflect the evolutionary differentiation between and within the continental groups. Cuba represents the evolutionary centre in the New World with the highest number of species and the greatest morphological and phylogenetical diversity, from where only few phyletic groups seem to have migrated to the other Antilles and Central America.

Key words: *Buxus*, taxonomy, pollen, evolution.

INTRODUCTION

The *Buxaceae* is a small family with four or five genera and approximately 120 species of mostly woody, rarely herbaceous plants.

Buxaceae are frequently associated with Euphorbiaceae and variously assigned to Celastrales. Alternative placements are in, or close to, Hamamelidales, as separate order in Takhtajan's (1987) Hamamelididae and within Pittosporales (Thorne, 1989), or as an order of its own in Dahlgren's (1980, 1983) Rosiflorae. The preponderance of evidence points towards a phylogenetic position near the base of the non-magnoliid dicotyledons between hamamelid and dilleniid groups. Results of DNA sequence analysis place *Pachysandra* within the hamamelids, as sister of Trochodendrales, with relationships to Sabiaceae and Proteaceae (Chase & al. 1993, von Balthazar & al. 2000).

The family obviously represents an ancient group. There are early fossil records (*Spanomera*) from Albian/Cenomanian of the Potomac Group suggesting a close relationship to the lineage of *Buxaceae* (Drinnan & al., 1991). The 'crotonoid' *Pachysandra-Sarcococca* pollen is first reported from the Upper Cretaceous of Central Europe. Colporate *Buxus* pollen is known from the Lower Eocene (Kedves, 1962), followed by a succession of types (Bessedik, 1983), confirming the coherence with pantoporate *Buxus* pollen of the Lower Miocene (Kruttsch, 1966).

Classification of the Genus

The largest genus has a disjunct intercontinental distribution with one centre of variety in the Caribbean-Central American region with about 50 species, originally described as genus *Tricera* (Schreber, 1791) and later on regarded as section *Tricera* of the genus *Buxus* (Baillon, 1859).

Another centre of distribution is found in East Asia, where section *Buxus* comprises about 40 species occurring from China, Korea and Japan to Malaysia, the Philippines and Indonesia, including the Mediterranean and North African *taxa* as most western representatives. Six species from tropical Southeast Asia are delimited as section *Eugeniobuxus* (Hatusima, 1942).

A comparatively low number of seven or eight species occurring in Africa and Madagascar. In a recent synoptic revision Schatz & Lowry II (2002) describe seven new species and report in total nine species for Madagascar, two of them occurring on the Comoros, too. The African-Malagasy species can be arranged in two different phyletic groups, originally described as genera *Buxanthus* and *Buxella* by Van Tieghem (1897), now regarded as subsections of the African section *Proboxus* (Mathou, 1940).

The genus has a wide ecological range, growing in dry scrub forests, on limestone cliffs, in the understorey of montane rain forests, occasionally in cloud forests, sometimes above 3 000 m; frequently settling on ultramafic

soils, where the species have developed the capacity of hyperaccumulating nickel (Reeves & al., 1996).

Anatomical Data

According to comprehensive character analysis, the African *taxa* show the most primitive characters. This can be demonstrated by the pattern of petiole and axis vascularization pointed out by Van Tieghem (1897) already (Fig. 1).

Whereas the African *B. hildebrandtii* group has three vascular bundles in the petiole and two pairs of cortical bundles in the axis (Fig. 1C), the latter are absent in all neotropical species (Fig. 1A). In contrast, the majority of the Eurasian *taxa* have only a median vascular bundle and two lateral sclerenchyma traces in the petiole, and a cortical vascular bundle in each angle of the branchlets, accompanied by one to two fibre strands (Fig. 1B). (Van Tieghem, 1897; Mathou, 1940).

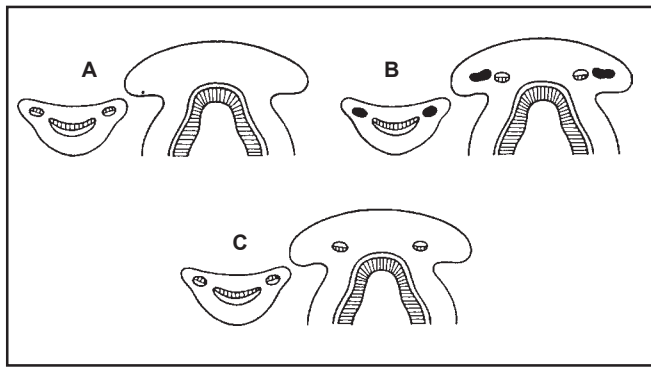


Fig. 1. Vascularization pattern of petiole and stem cross sections. A. sect. *Tricera* (American species); B. sect. *Buxus* (Eurasian species); C. sect. *Probuxus*, subsect. *Buxanthus* (North East African species). (after Mathou, 1940).

Thus, only the East African *B. hildebrandtii* group possesses the complete original configuration of the *Buxus* progenitors, from which the American, the Eurasian and the South African *taxa* could have evolved independently along different lines.

Palynological Data

These considerations are supported by pollen morphological correlations. The basic pollen type of *Buxus* is a sphaeroidal 3-colporate grain with long colpi, each containing 3-4 to 6 circular ora. A peculiar configuration which has to be regarded as an ancient aperture type of dicotyledons (Köhler, 1981). Starting from this basic type a trend towards an increase in number of colpi, accompanied by transitions from zonal to global arrangement, leads to 6-9-12 pantocolporate grains. Shortening of ectoapertures combined with reduction of number of ora finally gives rise to 12-40-pantoporate grains (Fig. 2). This pattern of aperture evolution occurs in 4 pollen types of the New World species and reappears with identical successions in the African ones, while the East Asian species possess the high evolved pantoporate state only.

The exine sculpture displays a broad variation, providing taxonomically significant characters (Fig. 3). In the American species it comprises comparatively free, interlaced ridges, partly merging ridges and a coarse or fine reticulum with undulating muri, which sometimes bear small spinulae. Others have supratectal pilate-verrucate and transversely ribbed elements.

The Eurasian *taxa*, phylogenetically well separated, present a striking similarity in exine ornamentation with the American species, while the South African-Malagasy *taxa* have reticulate-pilate to verrucoid exine sculpture recalling the pattern of some of the Asian species (Köhler, 1994). The exine pattern of the East African *B. hildebrandtii* finally reminds one again of some of the Caribbean species.

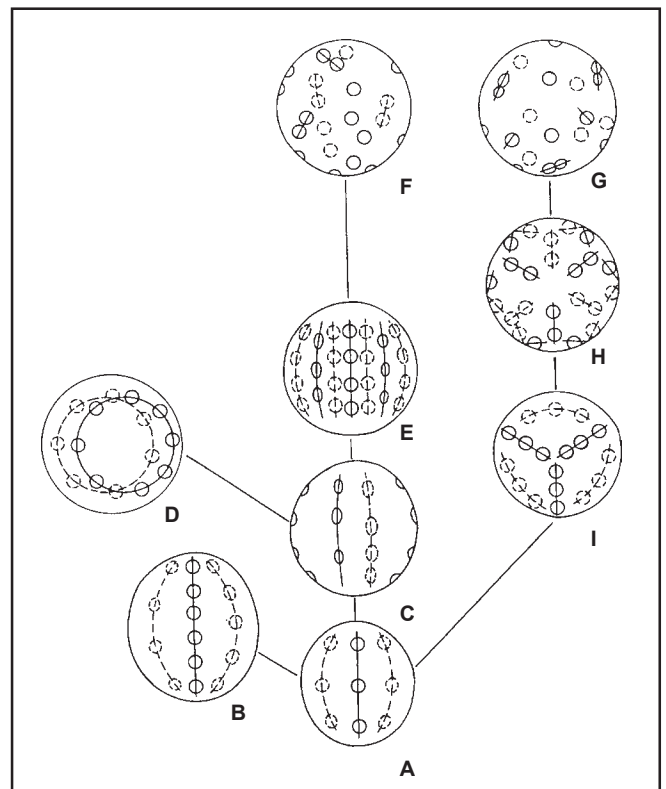


Fig. 2. Pollen aperture evolution of the American *Buxus* species. A-B. tricolporate grains with varying numbers of ora per colpus. C, E. 4-7-colporate grains, colpi in zonal position with varying numbers of ora. D. 2-syncolporate grains, a rare case. G-H. 6-antocolporate to 12 pantocolporate grains with reduced numbers of ora. I, F. pantoporate grains, occasionally with short colpus-rudiments.

It is fascinating how in clearly separated phyletic groups from different continents nearly the whole array of exine sculpture variation is achieved. We are obviously concerned with an example of parallelism, the independent occurrence of similar changes in groups of common ancestry and because they had a common ancestry (Simpson, 1961). It seems to be the expression of a common ancestral genetic basis, validating the common descent of the different phyletic groups and speaking in favour of keeping them together in a single genus *Buxus*.

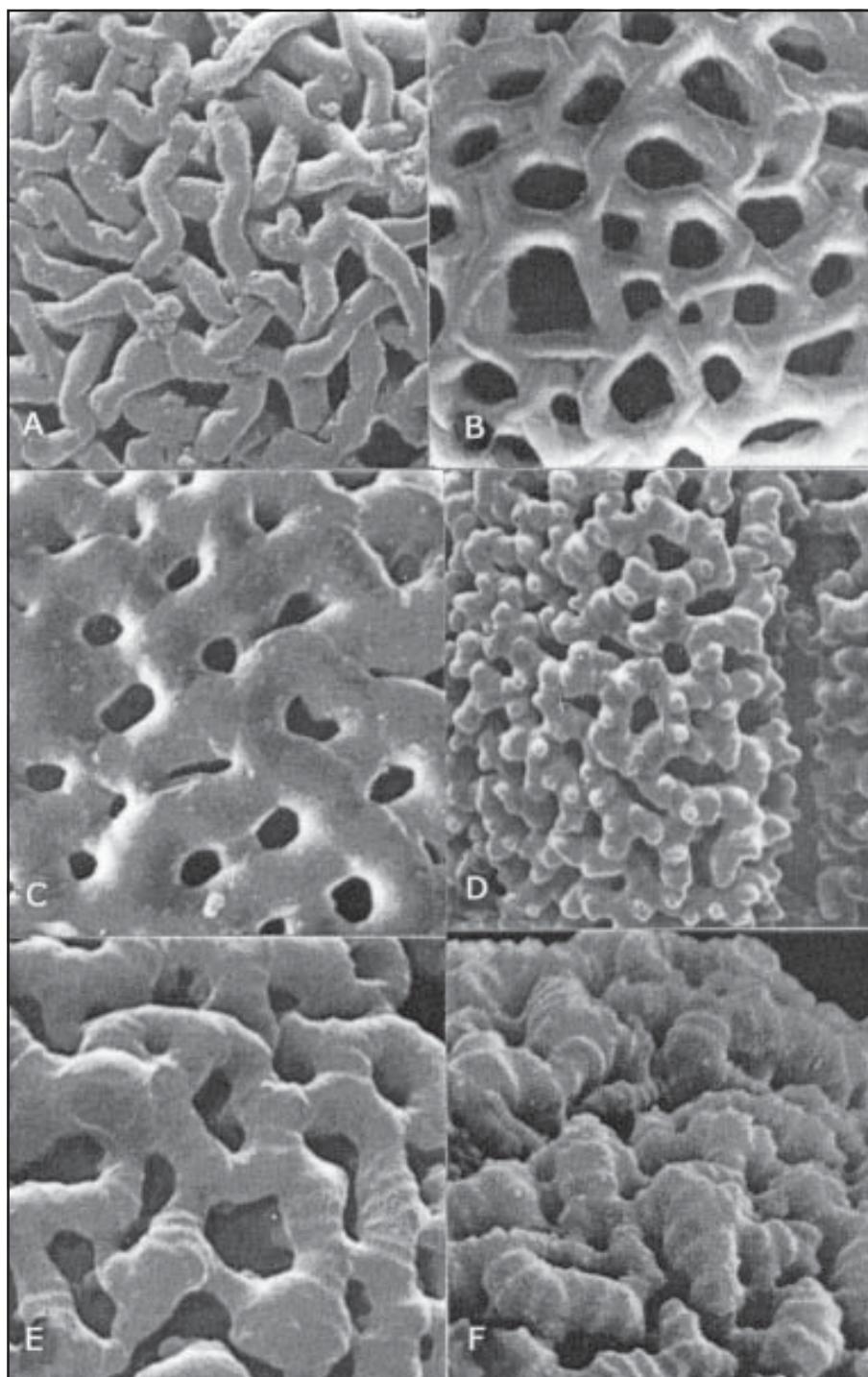


Fig. 3. Exine sculpture of American taxa. A. Comparatively free, interlaced and anastomosing ridges (*B. arborea*, 9500 X), B. condensation and increasing fusion of the ridges (*B. aneura*, 7000 X), C. elevated murus segments thicker than the lower ones (*B. pilosula*, 9500 X), D. undulating muri bear small spinulae (*B. foliosa*, 7000 X), E. muri in part strongly thickened (*B. vahlii*, 9000 X), F. muri verruca-like enlarged and transversely ribbed (*B. retusa*, 9500 X).

Leaf Venation

The evolutionary differentiation of these groups on various continents is well reflected by the patterns of leaf venation (Fig. 4).

The basic leaf venation is a brochidodromous pattern with secondaries secundar joined together in a series of arches

forming an intramarginal vein. This type is found in all the New World species, which can be arranged in further subgroups (Fig. 4, A-E), (Köhler, 1984). It appears again in the East African *B. hildebrandtii* with a great similarity to the *B. shaferi* group from East Cuba (Köhler, 1985). Among the Asian species this pattern appears once again in tropical species from Thailand, Viet Nam and Malaysia (Brückner, 1993).

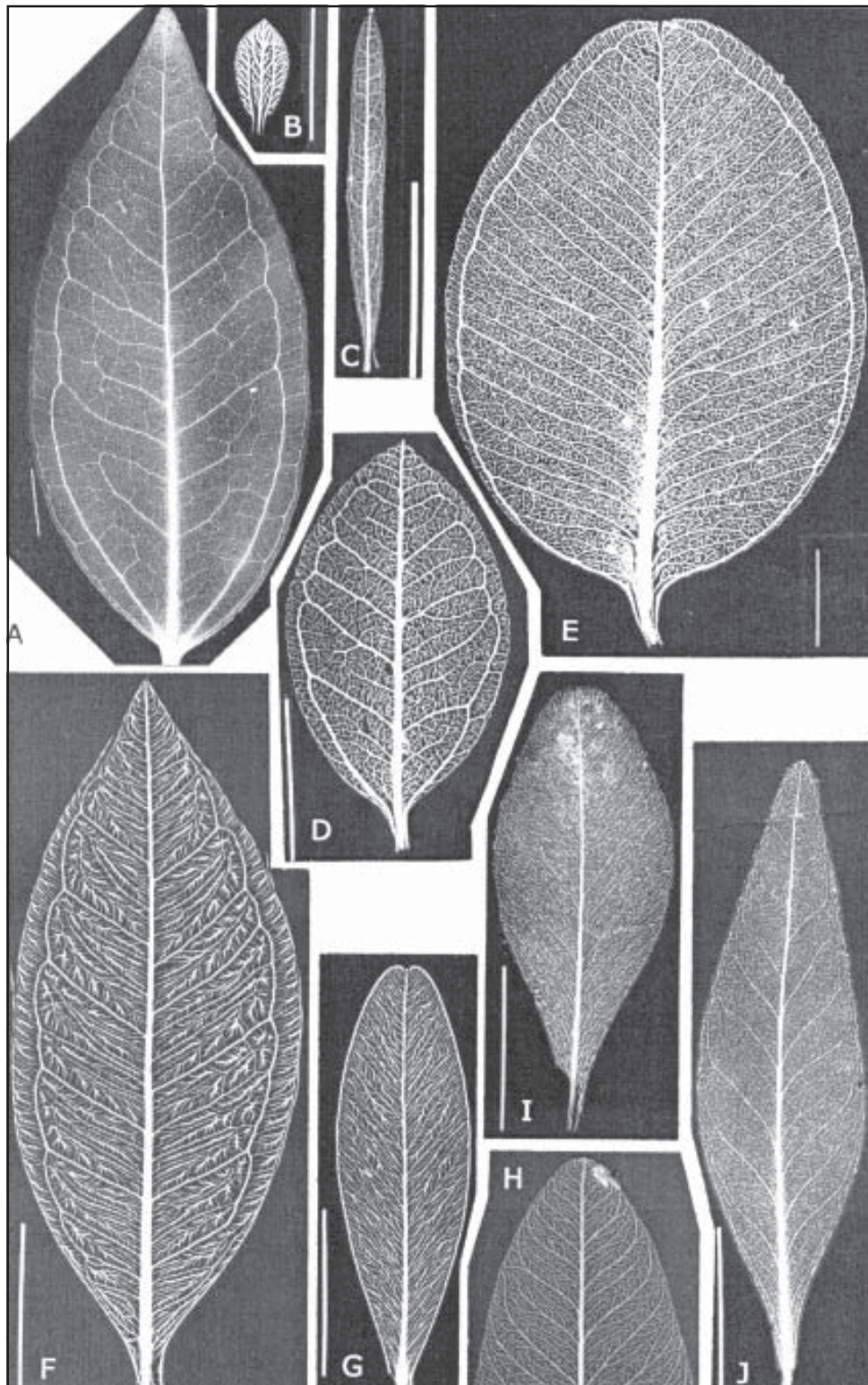


Fig. 4. Leaf venation pattern of the genus *Buxus*. A-E. American Sect. *Tricera*, brochidodromous: A- *B. citrifolia*, B- *B. vaccinioides*, C- *B. leoni*, D- *B. rheedioides*, E- *B. crassifolia*. F Sect. *Eugeniobuxus*, special type: *B. rivularis*, G-H. Sect. *Buxus*, specialized brochidodromous: G- *B. austro-yunnanensis*. H- *B. rupicola*, I-J. Sect. *Probuxus*, + cladodromous: I- *B. macowanii*, J- *B. madagascariensis*.

This brochidodromous venation type has a disjunctive distribution from the Caribbean over East Africa to Southeast Asia and seems to reflect an ancient Tethyan constellation, from which various groups have derived. By

reduction of leaf size, in connection with adaptations to arid and temperate climates, the intramarginal vein becomes dislocated to the utmost leaf edge and the secondaries branching predominantly exmedially giving rise

to a specialized brochidodromous pattern (Fig. 4, F-G-H). The *taxa* belonging to this type have experienced a remarkable adaptive radiation in East Asia, forming an important centre of diversity, extending westward to the Mediterranean area with *B. sempervirens* and *B. balearica*. Among the African-Malagasy species *B. madagascariensis* is the most primitive with a loosely brochidodromous to eucamptodromous venation (Fig. 4, I), from which the South African species around *B. macowani* can be derived with completely reduced arch segments, its secondaries branching once or more towards the edge forming a cladodromous pattern, without intramarginal vein (Fig. 4, J), (Köhler & Brückner, 1990).

With the most primitive characters the East African *B. hildebrandtii* represents a relic group reminding of the early precursors of the genus. It is the only Old World taxon that stands closest to the progenitors of the New World *taxa* (Köhler 1985). A further example for an ancient disjunction —Tropical America/East Africa— known from several other genera e.g. *Dracaena*, *Savia*.

Generally the northern border of the Tethys is considered as migration route (Madrean-Tethyan links), but the present case seems to speak in favour of the southern Tethyan coasts with its rich laurophyll vegetation existing up into the Miocene (Axelrod, 1975).

So, *Buxus* may belong to those genera that Borhidi (1985), calls “periocean”, formerly inhabiting a broad coastal zone of these continents. It seems probable that besides the Macaronesian Plate parts of the Caribbean Plate could have been involved in this area.

Cuban Buxus

The American *Buxus* possess their most important centre of diversity with more than 35 species in Cuba, where they have undergone an extraordinary adaptive radiation. In Cuba, not only do those *taxa* closest to the East African ones exist, but also nearly all the other relationship groups are present. Especially the ancient mountain systems in East Cuba appear as centres of origin, from where often only a single branch migrated in different extent to the other Antilles and to Central America (Köhler, 1984, 1985, Köhler & Brückner, 1990).

The exine sculpture of the pollen grains lends important characters for the recognition of relationship groups and their migration routes in Cuba and the Caribbean. Two examples:

The type with comparatively free, interlaced ridges comprises the Cuban *B. acuminata*, *B. bissei*, *B. rheedioides*, *B. sclerophylla*, *B. wrightii* and *B. leoni*. It obviously migrated to the geologically younger Jamaica where in total only four to five species exist, *B. arborea*, *B. macrophylla*, *B. purdieana*, belonging to this type.

Only *B. laevigata*, which could not be localized in Jamaica since Swartz's type collection, possesses a different pollen type, which might support the doubts about the provenance of Swartz's specimen.

As another example I want to mention the species around *B. glomerata*-group, having a distinct reticulate exine pattern with small spinules.

With *B. shaferi*, *B. baracoensis*, *B. obovata*, *B. moana*, *B. foliosa*, *B. pseudaneura* and *B. leivae* they have experienced an adaptive radiation on serpentine soils mainly in the Sierra de Moa. In contrast, another branch with glabrous gynoecium with *B. jaucoensis*, *B. yunqueensis* and *B. glomerata* has achieved various adaptations to limestone rocks. All the *taxa* concerned have a great variety in leaf size and form, leaf anatomy, especially secretorial structures and SEM surface pattern, which are very useful for species delimitation and clearly demonstrate the manifold adaptive strategies, important for the evolutionary differentiation. The *taxa* settling on limestone are younger and higher evolved. Besides, *B. glomerata* is the only species that Hispaniola shares with Cuba.

Of the remaining Antilles Puerto Rico has two species with relations to Cuban groups, Martinique one and the Bahamas share one with Cuba and some other islands. Five species are distributed in Central America and Mexico, and so far, one species reported from Venezuela has also been found in Panama. There are further new records from Colombia and from Surinam.

Most of the more than 35 Cuban species grow in particular areas only, and the small populations possess a restricted distribution, liable to extinction. Nearly one third (31%) of the species are classified in some endangered category according to IUCN (1994) and referring to recent evaluations eight species have been categorized Critically Endanger.

During a comprehensive excursion programme (1995-2001-2003), sponsored by Braimbridge, Langley Boxwood Nursery London and in part by the American Boxwood Society, most of the species (95%) could be located, often in very remote, hardly accessible areas, and are now cultivated in the green house of the National Botanic Garden. So far 35 species are in culture and some more are still in investigation.

The field work offered unique chances to study the species in their natural habitats, to gain informations about population structure, growth-form, seed production and propagation rate, about biological and ecological aspects of the biotopes including altitude, soil types and humidity. The National Collection of *Buxus* is a unique example for a comprehensive living gene bank, providing an excellent base for the

preservation of the extremely rich genetic heritage of *Buxus* in Cuba, for the protection and possible reintroduction of threatened species.

The collection offers an invaluable base for scientific research that can't be done in the natural habit. It can give support for the species rank of some critical taxa (Köhler, 1998) and it offers important data for the taxonomic revision in progress for the new Flora of Cuba. In that context it will contribute fundamentally to the understanding of the highly interesting aspects of the evolution of the Cuban *Buxus* species and its differentiation in the Caribbean and Central America.

The small flowers of *Buxus* obviously are pollinated by very small insects having an extremely reduced activity space, which becomes further limited by permanent winds forcing the insects to keep within the bushes. These conditions facilitate reduction or interruption of gene exchange, furthering differentiation and isolation of new species. Speciation often occurred within limited areas, a mountain or a valley system, showing parallel evolution to those of the neighbouring systems, giving rise to vicariant taxa.

Many species are close together, appearing as relatively young differentiations, supported by the geological findings that the present physiogeographical situation has been formed during the last 10 million years.

These conditions in connection with the high diversity of edaphic and climatic factors and with the turbulent geological history of the Caribbean obviously have created the evolutionary centre in Cuba.

BIBLIOGRAPHY

Axelrod, D. I. 1975. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. – *Ann. Missouri Bot. Gard.* 62: 280-334.

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

Balthazar von, M., Endress, P. K. & Qiut, Y. L. 2000. Phylogenetic Relationships in Buxaceae based on nuclear internal transcribed Spacers and Plastid *ndhF* Sequences. – *Int. J. Plant Sci.* 161: 785-792.

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

Batdorf, L. R. 1995. Boxwood handbook. American Boxwood Society, Boyce, USA.

Batdorf, L. R. 2004. Boxwood, An Illustrated Encyclopedia. The American Boxwood Society, Boyce, Virginia.

Bessedik, M. 1983. Le genre *Buxus* L. (Nagyipollis Kedves 1962) au Tertiaire en Europe occidentale: évolution et implications paléogéographiques. – *Pollen et Spores* 25: 461-486.

Borhidi, A. 1985. Phytogeographic survey of Cuba. 1. The phytogeographic characteristics and evolution of the Flora de Cuba. – *Acta Bot. Hungar.* 31: 3-34.

Brückner, P. 1985. Blattnervatur und Pollenmorphologie eurasiatischer Arten der Gattung *Buxus* L. (Buxaceae Dumort.) und ihre Bedeutung für die Systematik. Ph. D. Berlin.

Brückner, P. 1993. Pollen morphology and taxonomy of Eurasiatic species of the genus *Buxus* (Buxaceae). – *Grana* 32:65-78.

Chase, M. W., Soltis, D. E., Olmstead, R. G., Morgan, D., Les, D. H., Mishler, B. D., Duvall, M. R. & al. 1993. Phylogenetics of Seed Plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. – *Ann. Missouri Bot. Gard.* 80: 528-580.

Dahlgren, R. 1980. A revised system of classification of the angiosperms. – *Bot. J. Linn. Soc.* 80: 91-124.

Dahlgren, R. 1983. General aspects of angiosperm evolution and macrosystematics. – *Nord. J. Bot.* 3: 119-149.

Drinnan, A. N., Crane, P. R. & Friis, E. M., Raunsgaard Pedersen, K. 1991. Angiosperm flowers and tricolpate pollen of Buxaceous affinity from the Potomac group (Mid-Cretaceous) of Eastern North America. – *Am. J. Bot.* 78: 153-176.

Hatusima, S. 1942. A revision of the asiatic *Buxus* L. – *J. Dpt. Agricult. Kyusyu Imp. University* 6: 261-342.

Howard, R. A. 1963. Notes on *Buxus* L. in the Lesser Antilles and on Mathou's overlooked publication. – *J. Arnold Arbor.* 44: 96-100.

Kedves, M. 1962. Nagyipollis, a new pollenfgen. from the Hungarian Lower Eocene. Szeged. – *Acta Biol.* 8: 83-84.

Köhler, E. 1981. Pollen morphology of the West Indian-Central American species of the genus *Buxus* L. (Buxaceae) with reference to taxonomy. – *Pollen et Spores* 23: 37-91.

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

Köhler, E. 1984. Zur Blattnervatur der neotropischen *Buxus*-Arten und ihre Bedeutung für die Systematik (Buxaceae). – *Flora* 175:345-374.

Köhler, E. 1985. Vorstellungen zur Evolution und Chorogenese der neotropischen *Buxus*-Arten. – *Feddes Repert.* 96:663-675.

Köhler, E., Schirarend, C. 1989. Zur Blattanatomie der neotropischen *Buxus*-Arten und ihre Bedeutung für die Systematik. – *Flora* 183:1-38.

Köhler, E. & Brückner, P. 1990. Considerations on the evolution and chorogenesis of the genus *Buxus* (Buxaceae). – *Mem. New York Bot. Gard.* 55:153-168.

Köhler, E. 1993. Blattnervatur-Muster der Buxaceae Dumortier und Simmondsiaceae Van Tieghem. – *Feddes Repert.* 104:145-167.

Köhler, E. 1994. Parallel evolution of pollen characters in the genus *Buxus* L. (Buxaceae). – *Acta bot. Gallica* 142:223-232.

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

Köhler, E. 2003. Buxaceae in Smith, N., Mori, S. A., Hendersen, A., Stevenson, D.W. & Heald, S. V. (ed.) Flowering Plants of the Neotropics. Princeton University Press, Princeton and Oxford:70-72.

Köhler, E. 2004. The National Collection of Native *Buxus* species in Cuba.— *Topiarius* 7:12-16.

Köhler, E. in print. Buxaceae in Kubitzki, K. (ed.) The Families and Genera of Vascular Plants. Springer.

Krutzsch, W. 1966. Zur Kenntnis der präquartären periporaten Pollenformen. — *Geologie* 15: 16-71.

Mathou, Th. 1940. Recherches sur la famille des Buxacées. Les Frères Douladoure. Toulouse.

Reeves, R. D., Baker, A. J. M., Borhidi, A. & Berazaín, R. 1996. Nickel-accumulating plants from the ancient serpentine soils of Cuba. — *New Phytol.* 133: 217-224.

Simpson, G. G. 1961. Principles of Animal Taxonomy. New York and London.

Schatz, G. E., Lowry II, P. P. 2002. A synoptic revision of the genus *Buxus* L. (Buxaceae) in Madagascar and the Comoro Islands. — *Adansonia* ser.3 24: 179-196.

Takhtajan, A. L. 1987. *Systema Magnoliophytorum*. 1. Leningrad: Izd. Nauka.

Thorne, R. F. 1989. 'Hamamelididae' a commentary. In: Crane, P. R., Blackmore, S. (Eds.). *Evolution, systematics and fossil history of the Hamamelidae*. Vol. 1. Clarendon Press. Oxford.

Tieghem van, P. 1897. Sur les Buxacées. — *Ann. Sci. Nat., Bot. Sér.* 8,5: 289-338.

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