Investigation on occurrence and ultrastructure of the proteinaceous nuclear inclusions (PNIs) in the Bignoniaceæ, with special reference to the geographic distribution patterns

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Investigation on occurrence and ultrastructure of the proteinaceous nuclear inclusions (PNIs) in the *Bignoniaceæ*, with special reference to the geographic distribution patterns

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SUMMARY — This paper analyzes the occurrence and distribution of Proteinaceous Nuclear Inclusions (PNIs) in the family Bignoniaceæ. In 52 out of 70 examined species (corresponding to 74.2%) nuclear inclusions with lamellar substructure are observed. An additional crystalline body, derived by packing of lamellar inclusion subunits during cell development, is reported in several taxa. PNIs are recorded in all studied tribes, but with different distribution. Most Tecomeæ and all taxa of Crescentieæ, Coleeæ, Oroxyleæ, Eccremocarpeæ and Schlegeliæ show PNIs. In most Bignonieæ they are lacking. The occurrence of PNIs in two tribes considered to be the oldest groups of Bignoniaceæ and related to the ancestral stock of Tubifloræ, Tecomeæ and Oroxyleæ, support their presence as an ancestral trait. Although systematic conclusions in Bignoniaceæ cannot to be made in consequence of the great structural homogeneity of PNIs, the relationships between occurrence data and geographic distribution patterns, are discussed. Finally, the placement of *Schlegelia* and *Paulownia*, regarded as transitional genera between Bignoniaceæ and Scrophulariaceæ, are analyzed.

INTRODUCTION

Bignoniaceæ Juss. is a family of woody trees, shrubs or vines, rarely herbaceous, mostly tropical with a few genera in warm temperate regions. Since the last century, the taxonomic treatment of the family has been controversial and only recently it has been divided into eight tribes consisting of 113 genera and 800 species (GENTRY 1980).

Tecomeæ is found in both the Old and the New World and it represents perhaps the oldest extant group of Bignoniaceæ (GENTRY 1974, 1980). Bignonieæ s. str., Crescentieæ s. str., Tourrettieæ, Eccremocarpeæ and Schlegelieæ are restricted to the New World, Oroxyleæ is localized in Southeast Asia and Coleeæ is mainly centered on Madagascar with a single monotypic genus *(Kigelia)* in continental Africa.

Accentuated phenomena of parallelism and convergence in useful taxonomic characters make any systematic considerations very difficult. For instance,

indehiscent fruits evolved separately in at least two different lineages (GENTRY 1976) and nearly all of the highly diversified pollen types of the family have evolved independently in unrelated groups (FERGUSON and SANTISUK 1973; BUURMAN 1978; GENTRY and TOMB 1979; HARLEY and BANKS 1993; BOVE 1993). On the other hand, data from cytology (GOLDBLATT and GENTRY 1979) or chemotaxonomy (SCOGIN 1980, 1985; SATYAVATHI *et al.* 1987) have been demonstrated to be remarkably constant and, though they have contributed to better knowledge of the family, the leave many unsolved problems.

Still, the delimitation of the family is a open taxonomic question, especially regarding Scrophulariaceæ. The genera *Paulownia, Schlegelia, Gibsoniothamnus* and *Synapsis* have been placed, by various authors, in one family or the other or regarded as transitional genera between Bignoniaceæ and Scrophulariaceæ (URBAN 1926; CAMPBELL 1930; WESTFALL 1949; MONACHINO 1949; WILLIAMS 1970; ARMSTRONG 1985). GENTRY (1980) regards *Paulownia* as an intermediate genus linking these two families, while he considers the other three genera anomalous, showing no clear relationship to other members of the family and, accordingly, segregated as the tribe Schlegelieæ.

As the ultrastructural features of proteinic nuclear inclusions (PNIs) in chlorenchyma cells have repeatedly been used as an additional tool of systematic value, especially for the circumscription and putative phylogenetic arrangement of Asteridæ families (SPETA 1977, 1979; BIGAZZI 1984, 1986, 1989, 1993*a*), an examination was conducted to determine the occurrence of PNIs within Bignoniaceæ and whether these could be utilized as a systematic character at the family level.

PNIs can appear amorphous, fibrillar, lamellar, crystalline or tubular and therefore they were classified in several different types and subtypes on the basis of their ultrastructural morphology. A detailed investigation of the fine structural organization of different forms of PNIs, elaborated by computer image treatment, shows that they consist of the same elementary subunits and therefore they correspond to different stage of structural complexity (BIGAZZI 1993*b*).

Although the nuclear inclusion function is still now unknown, its stability — and thus reliability as a systematic character — is shown by various evidence: *(a)* its basal structural uniformity, *(b)* its constant occurrence in phylogenetically related Angiosperm families and undoubtedly *(c)* the specificity of the PNIs as part of a genetic program expressed during cell differentiation.

MATERIALS AND METHODS

84 specimens corresponding to 70 species and 35 genera of Bignoniaceæ were investigated (Tab. 1 for identification and origin of the material). Living material is a prerequisite in ultrastructural research with the trasmission electron microscope

TABLE 1 - Summarizing scheme the Bignoniaceæ investigated taxa, the occurrence and type of PNIs in chlorenchyma cells, and the source of material. The taxa are arranged according to GENTRY (1980). Abbreviations. Inclusion type: O = lacking, L = lamellar. Seeds B.G. means that the plants are grown in the Botanical Garden of Florence from seeds supplied by specified Botanical Gardens.

Taxa	Inclusion	Source of material
Tecomeæ		
Campsis grandiflora (Thunb.) Schum.	O	B.G. Florence
Campsis radicans (L.) Seem.	O	B.G. Florence
Campsis × tagliabuana (Viv.) Rehder	O	B.G. Hambury
Catalpa bignonioides Walt.	L	B.G. Florence
Catalpa bungei C.A. Meyer	L	B.G. Florence
Catalpa duclouxij Dode	L	seeds B.G. Kunming Yunnan China
Catalpa ovata Don	L	B.G. Hambury
Chilopsis linearis (Cav.) Sweet	L	B.G. Kew, B.G. Hambury
Fernandoa adenophylla (Don) Steen.	L	seeds B.G. Auroville India
Fernandoa magnifica Seem.	L	Kenya: Malindi
Incarvillea arguta Royle	L	B.G. Kew; B.G. Hambury
Incarvillea emodi (Lindl.) Chatterjee	L	B.G. Kew
Incarvillea olgæ Regel	L	B.G Hambury
Incarvillea sinensis Lam.	L	seeds B.G. Marburg
Jacaranda caerulea (L.) Juss.	O	seeds B.G. Habana Cuba
Jacaranda mimosifolia Don	O	B.G. Florence, B.G. Hambury
Markhamia lutea (Benth.) Schum.	L	B.G. Kew
Markhamia stipulata Seem.	L	seeds B.G. Habana Cuba
Markhamia zanzibarica (Bojer ex DC.) Schum.	L	B.G. Kew
Newbouldia leavis (Beauv.) Seem.	L	B.G. Kew
Pandorea jasminoides (Lindl.) Schum.	O	B.G. Florence; B.G. Hambury
Pandorea pandorana (Andr.) Steen.	O	B.G. Florence; B.G. Hambury
Podranea brycei (N.E.Br.) Sprague	L	B.G. Hambury
Podranea ricasoliana (Tanf.) Sprague	L	B.G. Florence; B.G. Hambury
Radermachera sinica (Hance) Hemsl.	L	seeds B.G. Queensland Australia
Radermachera xylocarpa (Roxb.) Schum.	L	seeds B.G. Auroville India
Rhigozum obovatum Burch.	L	B.G. Kew
Stereospermum kunthianum Cham.	L	Kenya: Malindi
Spathodea campanulata Beauv.	L	Kenya: Malindi, B.G. Florence
Tabebuia angustata Britton	L	seeds B.G. Habana Cuba
Tabebuia chrysotricha (Martius ex DC.) Stand.	L	seeds B.G. Rio de Janeiro
Tabebuia hypoleuca (Wright ex Sauv.) Urban	L	seed B.G. Habana Cuba
Tabebuia impetiginosa (Martius ex DC.) Stand.	L	B.G. Kew; seeds B.G. Rio de Janiero
Tabebuia lepidota (H.B.K.) Britton	L	seeds B.G. Habana Cuba
Tabebuia pallida (Lindl.) Miers	L	B.G. Kew
Tabebuia pentaphylla (DC.) Britton	L	B.G. Kew
Tabebuia rosea (Bertoloni) DC.	L	B.G. Florence
Tabebuia trachycarpa (Grisebach) Schum.	L	seeds B.G. Habana Cuba
Tecoma capensis (Thunb.) Lindl.	L	B.G. Florence; B.G. Hambury
Tecoma sambucifolia H.B.K.	L	B.G. Hambury
Tecoma stans (L.) Juss. ex Humb.	L	Kenya: Watamu; B.G. Hambury
Tecomanthe dendrophila (Bl.) Schum.	O	B.G. Kew
Tecomanthe venusta Moore	O	B.G. Kew

(continued)

TABLE 1 - *(continued)*.

Taxa	Inclusion	Source of material

Tecomeæ

Bignonieæ

Taxa	Inclusion	Source of material
Adenocalymma marginatum (Cham.) DC.	O	B.G. Kew
Anemopægma carrerense Armitage	O	B.G. Kew
Anemopægma chamberlaynii (Sims) Bur. & Schum.	O	B.G. Kew
Bignonia capreolata L.	O	B.G. Florence; B.G. Hambury
Clytostoma binatum (Thumb.) Sandw.	O	B.G. Kew
Clytostoma callistegioides (Cham.) Bur. ex Griseb.	O	B.G. Hambury
Distictis buccinatoria (DC.) Gentry	L	B.G. Hambury
Dolichandra cynanchoides Cham.	O	B.G. Rome
Macfadyena dentata Bur. & Schum.	L	B.G. Kew
Macfadyena uncata (Andr.) Sprag. & Sandw.	L	B.G. Kew
Macfadyena unguis-cati (L.) Gentry	L	B.G. Hambury
Mansoa difficilis (Cham.) Bur. & Schum.	O	B.G. Kew
Pithecoctenium crucigerum (L.) Gentry	L	B.G. Rome; B.G. Kew
Pithecoctenium cynanchoides DC.	L	B.G. Hambury
Pyrostegia venusta (Ker-Gawl.) Miers	O	B.G. Hambury
Saritaea magnifica (Steen.) Dugand	L	B.G. Florence

Crescentieæ

Taxa	Inclusion	Source of material
Crescentia alata Humb.	L	seeds B.G. Habana Cuba
Crescentia cujete L.	L	B.G. Florence
Crescentia mirabilis Ekman ex Urban	L	seeds B.G. Habana Cuba
Parmentiera aculeata (H.B.K.) Seem.	L	seeds B.G. Bogor
Parmentiera cereifera Seem.	L	B.G. kew

Coleeæ

Taxa	Inclusion	Source of material
Kigelia africana (Lam.) Benth.	L	Kenya: Malindi; B.G. Florence

Eccremocarpeæ

Taxa	Inclusion	Source of material
Eccremocarpus scaber Ruiz & Pav.	L	B.G. Kew; seed B.G. Marburg

Oroxyleæ

Taxa	Inclusion	Source of material
Millingtonia hortensis L.	L	seeds B.G. Auroville India
Oroxylum indicum (L.) Kurz	L	seeds B.G. Bogor

Schlegelieæ

Taxa	Inclusion	Source of material
Schlegelia parviflora (Oerst.) Monachino	L	B.G. Kew

Genera incerta sedis

Taxa	Inclusion	Source of material
Paulownia fortunei Hemsl.	L	seeds B.G. Kunming Yunming Yunnan China
Paulownia tomentosa (Thumb.) Steud.	L	B.G. Florence

(TEM). The plant material was rarely collected in the place of origin, but more commonly from plants cultivated in the Botanical Garden of Florence (= B.G. Florence), B.G. Hambury (Imperia), B.G. Rome, B.G. Kew and from plants grown under controlled conditions in the greenhouses of the Botanical Garden of the University of Florence from seeds supplied by Botanical Gardens all over the world.

Small pieces (2 × 2 mm) were cut with a razor blade from young, differentiated parts of leaves and immediately immersed in a fixative containing 4% glutaraldheyde in phosphate buffer 0.15M, at pH 7.2, for 1-3 hours at 4° C. Roots of *Tecoma stans* and cotyledons from seedlings of *Spathodea campanulata*, grown from seeds that were germinated on filter paper in Petri dishes, were fixed in the same way. The samples were then postfixed in 2% buffered osmium tetroxide for 3-4 hours at room temperature, dehydrated through a graded alcohol series and embedded in an Epon-Araldite mixture (FLUKA). Sections were obtained with a LKB IV ultramicrotome, placed on copper grids, stained with uranyle acetate and lead citrate and finally observed under a Philips EM 201-C working at 80 kV.

RESULTS

In 52 out of the 70 examined species of the family Bignoniaceæ (corresponding to 74.2%), proteinaceuos nuclear inclusions (PNIs) are a regular component of differentiated chlorenchyma cells (Table 1). Generally every nucleus contains only one nuclear body with lamellar structure, in close spatial association to the nucleolus (Figs. 1, 2, 3, 4, 5 and 7). These large inclusions are formed of superimposed filament layers in orderly arrangements, or laminæ, 11.0-12.0 nm thick (Fig. 2). In cross-section, the filaments in each lamina appear as rows of dots, and the square packing highlights the precise superimposition with the filaments of adjacent layers (Figs. 4 and 5). The different ultrastructural images of recorded PNIs, are the result of different angles of sectioning; the inclusions can appear as a paracrystalline body with a regular lattice pattern (Fig. 5) or as amorphous body discernible substructure (Fig. 3).

Data from Tab. 1 show a different distribution of PNIs in different tribes. The lamellar inclusion occurs constantly in Tecomeæ, Crescentieæ, Coleeæ, Eccremocarpeæ, Oroxyleæ and Schlegelieæ; on the other hand in a great many Bignonieæ genera they are lacking.

In the Bignoniaceæ PNIs are not exclusively localized in photosynthesing mesophyll cells, but they occur in different cellular types such as roots (Fig. 6) leaf epidermis, trichomes (Fig. 7) and cotyledonar leaves.

Numerous paracrystalline inclusions, in close spatial association to lamellar ones, are found in such taxa as *Tecoma capensis, Rhigozum obovatum* and in all *Incarvillea* species (Figs. 8, 9, 10, 11 and 12). These dense bodies commonly have polyhedral outlines and a periodic repeating substructure of about 10.5-11.5 nm parallel to the major axis of inclusion. Sometimes an unusual periodic structure of about 19.5-21.0 nm occurs transversely to the major axis, and

appears produced by the sliding of a part of the inclusion (Figs. 9 and 10). In these specimens the different sectioning planes give rise to a variety of «fish-bone» or «honey-comb» patterns (Fig. 11).

Ultrastructural observations on the biogenesis of these two inclusion types show a different time of deposition. In an early stage of cell development only lamellar bodies are formed and, later in the subsequent stages, the filaments of each lamella appear to aggregate together in a typical periodic crystalline structure (Fig. 12).

DISCUSSION

Bignoniaceæ show PNIs in a great variety of cell types. In addition to chlorenchyma cells, as in many Asteriadæ families (BIGAZZI 1984, 1986, 1989a, b, 1993a), they are found in epidermis, trichome, cotyledon and root cells. Structural details of PNIs in the Bignoniaceæ disclose a lamellar organization that appear to be formed of layers of parallel filaments, about 11.0-12.0 nm thick. It has recently been demonstrated that each filament is made up of two helically coiled fibrils measuring 3.0-3.5 nm in diameter (BIGAZZI 1993b). The paracrystalline bodies, observed in the developmental stages following the appearence of lamellar inclusions, are produced by aggregation of filaments in a more orderly crystalline type structure. A similar dynamic sequence was report-ed in the genus *Lavandula* of Labiatæ (MORASSI BONZI and BIGAZZI 1980).

Previously, lamellar nuclear inclusions in Bignoniaceæ were described in the gland cells of nectaries of *Catalpa bungei* (HEINRICH 1972). DUDEK and HESSE (1980) found in *Incarvillea variabilis* tubular inclusions similar to ones reported in *Linaria* (Scrophulariaceæ). Probably the inclusion, described by two

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Fig. 1-5 — Ultrastructure of lamellar nuclear inclusion in differentiated chlorenchyma cells.

Fig. 1. — *Parmentiera cereifera* Seem.: nucleus showing the close spatial relationship between lamellar inclusion (L) and nucleolus (nu). × 14,400.

Fig. 2. — *Pithecoctenium crucigerum* (L.) Gentry: longitudinal section of typical lamellar inclusion formed of superimposed filaments layers. × 45,500.

Fig. 3. — *Oroxylum indicum* (L.) Kurz: oblique section of lamellar inclusion without discernible substructure. × 37,300.

Fig. 4. — *Schlegelia parviflora* (Oerst.) Monachino: cross-section of lamellar body; the filaments in each lamina appear as rows of superimposed dots. × 69,500.

Fig. 5. — *Kigelia africana* (Lam.) Benth.: high magnification of lamellar inclusion in cross-section characterized by an square-lattice packing. × 204,000.
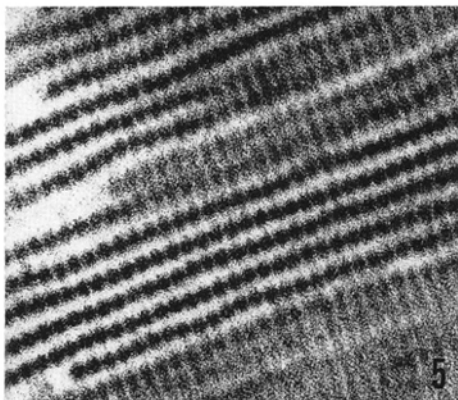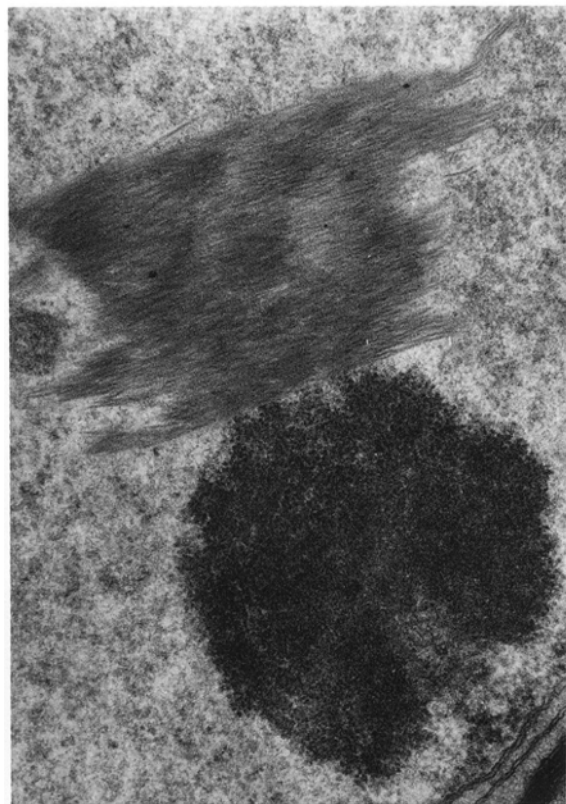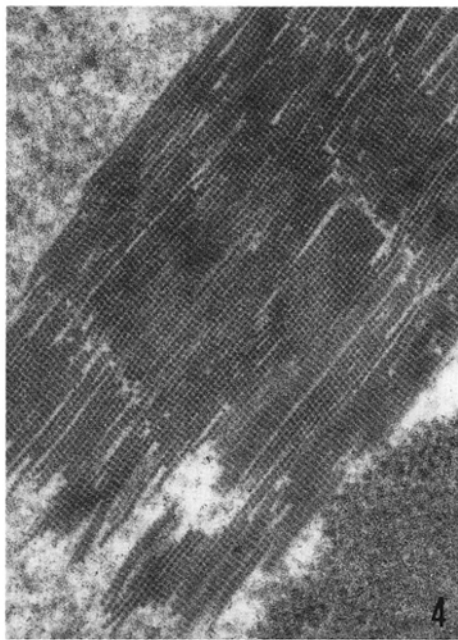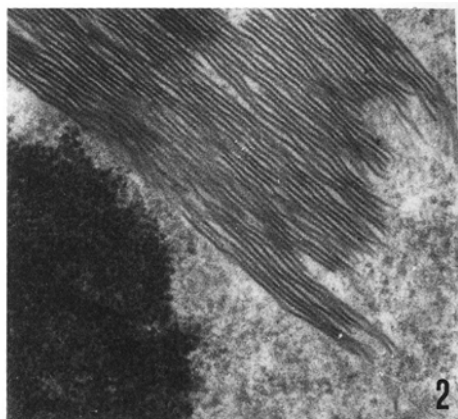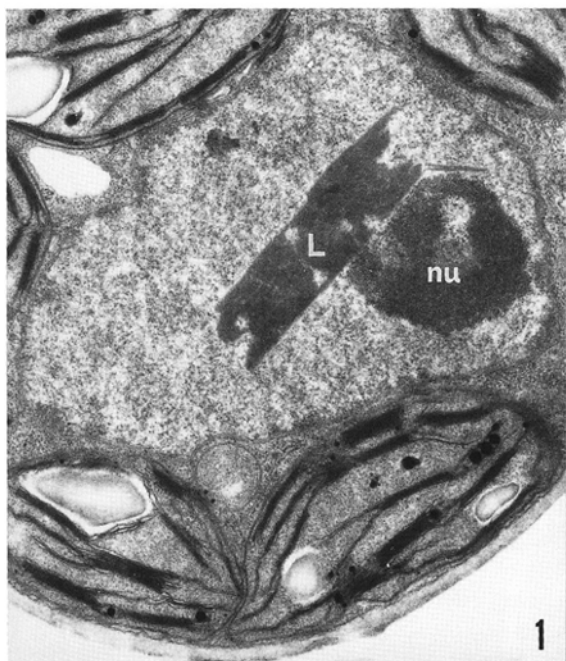
authors, corresponds to a paracrystalline body formed of closely packed la-mellæ. Infact the inclusion in *Incarvillea* is not made up of tubules and does not show the high degree of spatial organization present in that of *Linaria* (CIAMPO-LINI *et al.* 1980; BIGAZZI 1989*b*).

The great structural homogeneity of the PNIs observed in the Bigno-niaceæ, different from that reported in the allied family Scrophulariaceæ (BIGAZZI 1993*a*), makes any systematic consideration rather difficult. Neverth-eless, some suggestions on presence/absence of nuclear inclusions related to the distribution patterns of examined genera, can be made.

Analysis of the data summarized on the map of Tab. 2 shows a nonhomo-geneous occurrence of PNIs in the seven examined tribes. Data from only one tribe, the New World monogeneric Tourrettieæ, are lacking.

Bignonieæ is the largest tribe of the family. All genera are exclusively or predominantly vines or lianas, characterized by fruit dehiscence generally parallel to the septum. Most studied genera of this natural tribe, restricted to the New World, are devoid of PNIs (7 out of 11 genera), except for *Distictis*, *Macfadyena*, *Pithecoctenium* and *Saritæa* where lamellar inclusions in the nuclei of chlorenchyma cells were recorded.

Cosmopolitan Tecomeæ is the second major tribe of Bignoniaceæ. Most genera are trees, shrubs or rarely vines with fruit dehiscence perpendicular to the septum. PNIs show a different trend compared to Bignonieæ, as in 13 out of 17 investigated genera inclusions occur. Among the genera devoid of PNIs should be emphasized that *Tecomanthe* belongs with *Pandorea* to a distinct, closely related, circum-Pacific group of lianas, including *Campsis* in East Asia and North America, all sharing a similar shape of flower and fruit (VAN STEENIS 1978). In all African genera of Tecomeæ PNIs occur. This is particularly


Fig. 6. — *Tecoma stans* (L.) Juss. ex Humb.: part of young root tissue. Each nucleus contains only one, rarely two, lamellar inclusion in close spatial relationship to the nucleolus. × 4,900.

Fig. 7. — *Spathodea campanulata* Beauv.: longitudinal section of the leaf surface showing a grandular trichome formed by a pluricellular head, one stalk cell and two basal cells. Every nucleus of head cells contains a lamellar inclusion. × 2,900.

Fig. 8. — *Rhigozum obovatum* Burch.: lamellar inclusion (L) in a chlorenchyma cell nucleus closely connected with paracrystalline bodies (C). × 14,400.

Fig. 9. — *Incarvillea arguta* Royle: large crystalline inclusion in differentiated chlorenchyma cell nucleus that appears composed of several smaller bodies with two different periodic patterns. × 70,000.

Fig. 10. — *Incarvillea arguta* Royle: higher magnification of previous micrograph showing the two periodic structures of about 10.5-11.5 nm and 19.5-21.0 nm respectively. × 148,000.
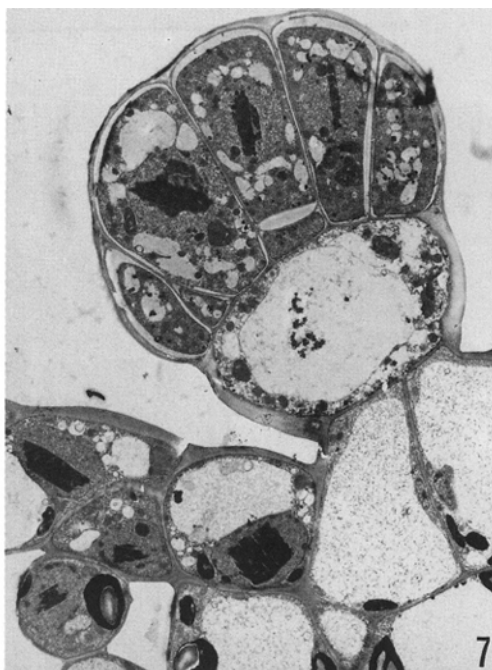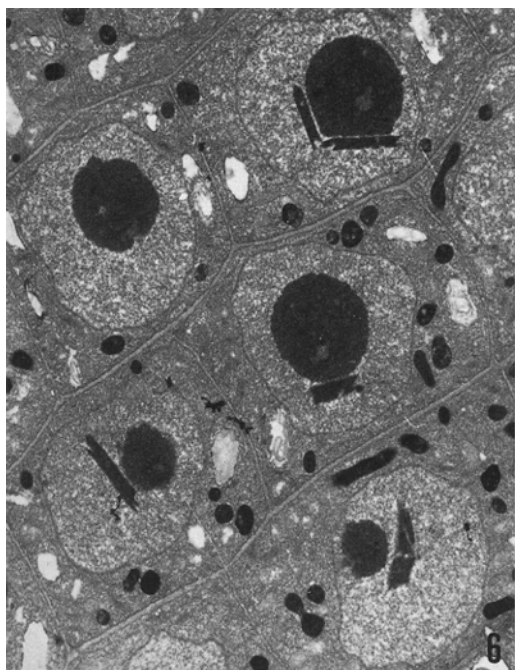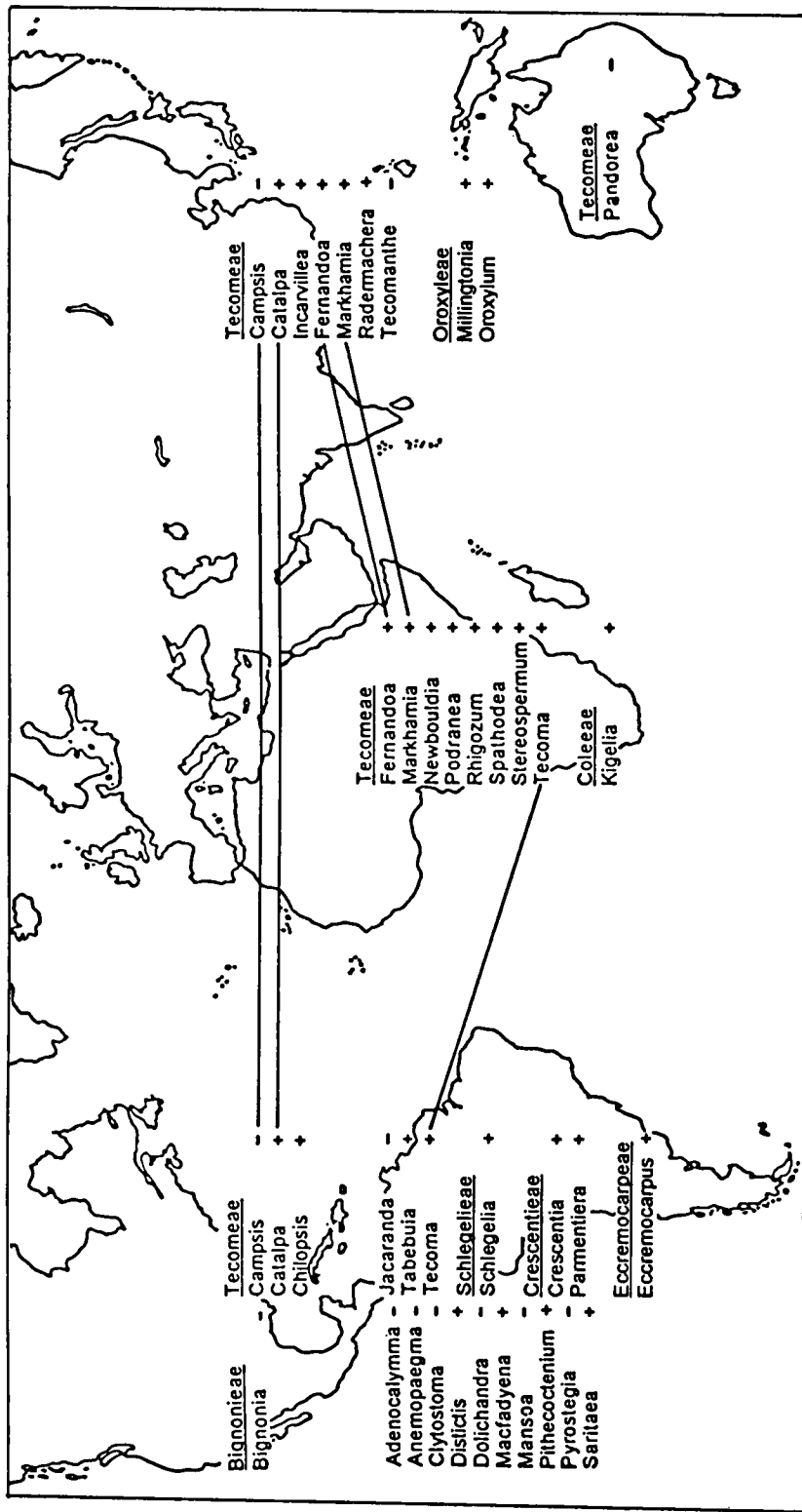
TABLE 2 - Map showing the distribution patterns of Bignoniaceæ genera and corresponding PNIs. The continuous lines emphasize the disjunct distribution of several investigated taxa.
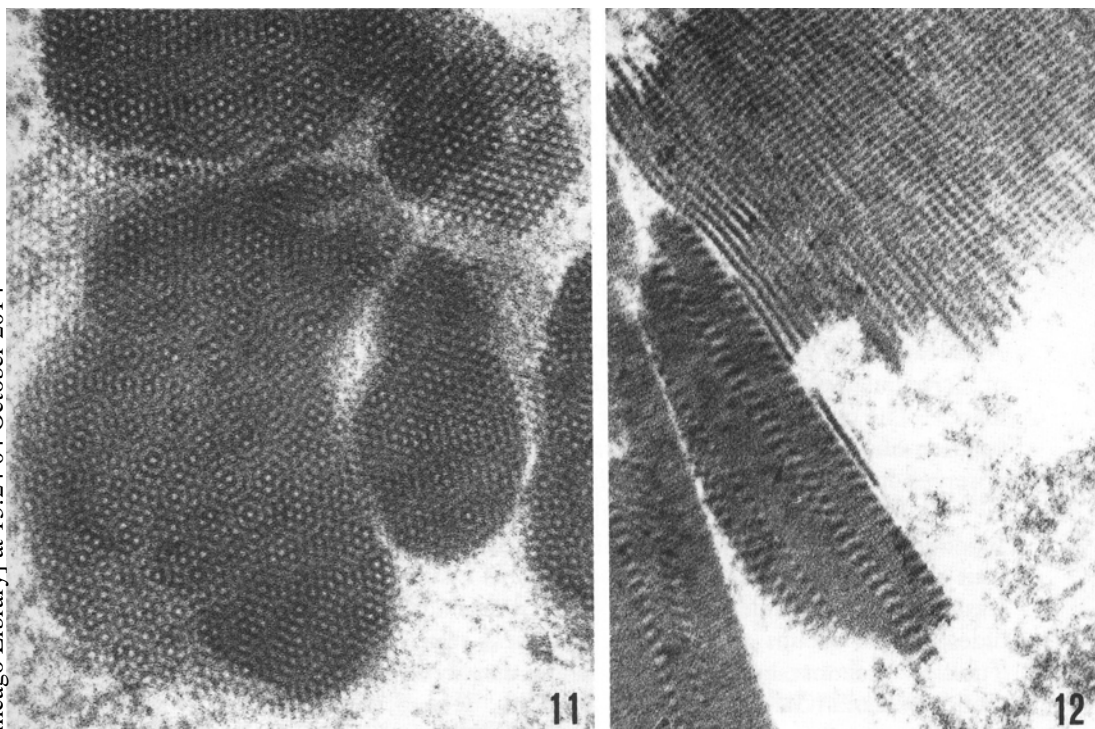
Fig. 11. — *Incarvillea sinensis* Lam.: different sectioning planes of crystalline inclusion in chlorenchyma cell nucleus showing a «fish-bone» or a «honey-comb» patterns. × 70,000.

Fig. 12. — *Tecoma capensis* (Thunb.) Lindl.: portion of cell nucleus with lamellar and crystalline inclusions close to each to other revealing aggregation of lamellæ in a periodic crystalline structure. × 93,000.

interesting in disjunct genera separated by the Atlantic (with one part of the distributional area in Africa and the other in South America) like *Tecoma*, or disjunct across the Indian Ocean (with one part of the area in Africa and the other in East Asia) like *Fernandoa* and *Markhamia*. Such a distribution consistency is a further evidence of PNIs specificity as part of a genetic program.

Crescentieæ and Coleeæ are differentiated from allied Tecomeæ by large indehiscent fruit. Trees or shrubs, most species have highly restricted distributions. Crescentieæ includes only three genera, distributed in southern Mexico, central America and northern South America. Coleeæ, consisting of six genera, is localized on Madagascar except for a single monotypic genus *Kigelia* in continental Africa. In all examined entities of Crescentieæ and Coleeæ lamellar

nuclear inclusions occur. The same situation was found in *Eccremocarpus*, the only genus of Eccremocarpeæ, a herbaceous vine localized in the Central Andes and in *Oroxylum* and *Millingtonia*, belonging to the new tribe Oroxyleæ (GENTRY 1980), which includes those Old World tree genera with fruit dehiscence parallel to the septum previously placed in Bignonieæ.

Finally *Schlegelia* and *Paulownia* appear to be closer to Scrophulariaceæ than Bignoniaceæ, although they are completely unrelated. *Schlegelia* is now placed in the exclusively neotropical tribe Schelegelieæ of Bignoniaceæ (GENTRY 1980) whereas the placement of *Paulownia* is still debated. *Paulownia* is sometimes allied with *Catalpa* in the Tecomeæ (AIRY SHAW 1966), but ARMSTRONG (1985) has shown that *Paulownia* has ovary characteristics typical of Scrophulariaceæ, while *Catalpa* clearly shows character states of Bignoniaceæ. The occurrence of lamellar inclusions does not clear up the systematic position of *Schlegelia* and *Paulownia* since the same type of inclusion has been observed in the majority of the tribes of Scrophulariaceæ (BIGAZZI 1993*a*).

As PNIs are recorde, although not uniformly distributed, in all tribes of Bignoniaceæ we could be justified in regarding their occurrence as an ancestral trait and not as an advanced character. This assumption is corroborated by at least two other considerations. First, Tecomeæ and Oroxyleæ appear to be the oldest extant groups of Bignoniaceæ closest related to the ancestral stock of Tubiflorae, almost certainly with wide distribution before the breakup of Gondwanaland (GENTRY 1974, 1976, 1980). Second, the occurrence of nuclear inclusions is a character shared with many families of Scruphulariales and more diffusely within Asteridæ (BIGAZZI, in prep.).

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