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Reproductive biology of *Phlogacanthus thyrsiflorus* Nees: An important medicinal plant of North-East India

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ABSTRACT

Phlogacanthus thyrsiflorus Nees is an important medicinal plant of the family Acanthaceae. The present article recorded different reproductive parameters of this species including floral morphology, anthesis, pollen production, germination, viability, foraging behaviour of flower visitors and the meiotic system. The species generally flowers during December-February and mature fruits during March-April. Flowers open in between 5.00 to 6.00 hrs. A single flower produces an average of 59,702 pollen grains. The maximum pollen germination was found in 15 % sucrose solution supplemented with 100 ppm boric acid. Pollen viability was determined by using Tetrazolium test (TTC), Muntzing's mixture and Acetocarmine. Chromosome counts revealed that *Phlogacanthus thyrsiflorus* is diploid i.e., 2n =42.

Keywords : *Phlogacanthus thyrsiflorus*, Reproductive biology, Floral morphology, Meiotic system.

Medicinal plants are of enormous economic importance and they are used as raw materials for the extraction of active constitution in pure form, as precursors for synthetic vitamins and steroids (Liza *et al*. 2010). In the context of increasing global interest in medicinal plants as potential sources of new bioactive molecules (Cordell 2000, Dahanukar *et al*. 2000, Ji *et al*. 2009), the conservation of medicinal plants as well as the knowledge of their uses is vital for the future of human health care (Shrestha *et al*. 2011). The reproductive biology of flowering plants is important for understanding pollination and breeding systems, for conservation, and for determining barriers to seed and fruit set (Tandon *et al*. 2003). Reproduction is a natural means of increasing the number of individuals of the same species and is vital for its evolution and survival. It is the only life processes which ensure the perpetuation of life. The physiology of reproduction in most of the flowering plants is closely under the control of environmental factors (Taiz and Zeiger 2003). For successful cultivation and conservation of plants the detail knowledge of plant reproductive biology is required (Moza & Bhatnagar 2007). The environment in which an organism lives affects its reproductive success (Sedgley and Griffin 1989). Environment exerts considerable influence on flowering, pollen fertility, *in vitro* pollen germination and fruit setting in plants (Shivanna 2003). Owing to the increasing and immediate concern for augmenting food supply, knowledge on reproductive biology has been utilized.

Phlogacanthus thyrsiflorus Nees commonly known as "Titaphul" is a medicinal plant belonging to the family Acanthaceae. This plant is distributed throughout the subtropical Himalayas, upper Gangetic plain, Bihar, North Bengal and Assam. Due to its wide range of medicinal uses, a large number of phytochemical and pharmacological investigations have been undertaken but the biological characters of this plant have not been investigated in detail. The whole plant possesses immense medicinal property and is used in whooping cough and menorrhagia. Leaf juice is used in asthma, jaundice, dysentery, tuberculosis, malarial fever and in rheumatism (Kalita and Bora 2008). Inflorescence is used for vermicide and also as remedy for cough. According to Khanikar (2005), flowers of *P. thyrsiflorus* are antidote to pox, used in jaundice, prevents skin diseases like sores, scabies etc. In spite of its excessive use for medicinal purposes and wide distribution, population decline of this plant has been attributed to anthropogenic factors (over exploitation, over grazing, forest floor denudation) and to the plant's own biology, including poor seed set and low germination. So, there is an urgent need for its conservation and it can be achieved by understanding its reproductive biology (Moza and Bhatnagar 2007). Present paper is a part of the attempt made to understand the reproductive biology of *Phlogacanthus thyrsiflorus*.

MATERIALS AND METHODS

The present study was carried out on *P. thyrsiflorus* grown in Tripura University in natural conditions. Ten healthy plants were selected from each population and observations were made on a day-to-day basis in natural habitats on flowering phenology which includes habit, phenology, anthesis etc. Floral morphology was also studied with the help of hand lens and dissection microscopy. *In vitro* pollen germination was carried out in different concentration of sucrose (2 %, 5 %, 10 %, 15 %, 20 %, 25 %, and 30 %) alone and in combination with Boric acid [100 ppm + 2 %, 100 ppm + 5 %, 100 ppm + 10 %, 100 ppm + 15 %, 100 ppm + 20 %, 100 ppm + 25 %, and 100 ppm + 30 %]. The experimental set up was done as per the method of Shivanna and Rangaswamy (1993). Pollen morphology was studied following Acetolysis method as proposed by Erdtman (1960). The size of the pollen grains (for radio symmetric ones the diameter in the polar view, and for bilateral ones the polar and equatorial axes) was measured in

glycerine jelly (Wodehouse 1935) using standard ocular micrometer. The terminology used is in accordance with Erdtman (1952), Faegrie and Iversen (1964), Walker and Doyle (1975), Nair (1964).The pollen production study was carried out following the standard methods (Nair and Rastogi 1963, Mandal & Chanda 1981). The pollen viability was assed using 1 % TTZ test following the standard method proposed by Norton (1966). The pollen ovule ratio is the more accurate measures of reproductive success than the total pollen per flower or per plant (Shivanna & Jhori 1985). Meiotic chromosome study was done by fixing young flower bud in 1:3 acetic alcohol for 24 hours.

RESULTS AND DISCUSSION

Floral biology and pollination mechanism- *Phlogacanthus thyrsiflorus* bears large number of orange tubular flowers arranged in terminal racemes (Table 1). There are two stamens i.e. diandrous and the gynoecium is bicarpillary, syncarpous with bilocular ovary, long style and bilabiate stigma. Various floral-polymorphic features i.e., position of stamens along with style was recorded in different flowers of the same individual (Fig. 4). The flowers open at morning in between 5.00h- 6.00 h. During this stage the two anthers comes closer to each other. Between 8.00 h-9.00 h anther dehiscence took place. Flowering and anthesis time may depend upon physical, physiological and biochemical factors of a plant as well as on climatic conditions (Hamilton 1959, Davies 1969, Reis and Kostic 1976). During that stage different pollinator's viz., *Homoptera* sp., *Anoplolepis* sp. (Fig. 5) visited the flower. By 11.00 h the anther sacs become completely empty (Fig. 4). Initial observations revealed that insect visitation is very less throughout the day. Frequencies of visits were recorded continuously for two days (36 h of total observation).

After flower opening few insects, thrips and some members of Diptera visit the flowers for foraging. The thrips are capable to enter the flower even in bud stage. They feed on pollen and stigmatic exudates. They crawl through anthers to stigma and help in pollination. *Anoplolepis* sp. is the main pollinator.

In vitro* pollen germination-**Pollen germination and the growth of pollen tubes are, in principle, necessary for fertilization and seed formation in flowering plants. Studies on *in vitro* pollen germination and pollen tube growth are very useful for explaining the lack of fertility (Pfahler *et al*. 1997). The effect of sucrose on *in vitro* pollen germination of ***Phlogacanthus thyrsiflorus showed that the taxa required comparatively low sucrose concentration (15 %) for their optimal germination and boric acid to some extent also influence the percentage of pollen germination. But, the best result was obtained in 15 % sucrose solution supplemented with 100 ppm boric acid. *In vitro* pollen germination method is rapid, reasonably simple and most commonly used for assessing pollen viability (Bhowmik and Datta 2012). In the present study, it is observed that the addition of boric acid and sucrose solution results in the increase in the rate of pollen germination as well as pollen tube development. In

Table 1—Floral Characters of *Phlogacanthus thyrsiflorus*

S. No.	Parameters	Observations
1.	Inflorescence	Terminal or Axillary racemes
2.	Bract	2, linear, pubescent, greenish brown
3.	Calyx	5, polysepalous, hairy, brown in colour
4.	Corolla	Bilabiate, zygomorphic,orange in colour with red stripes
5.	No. of Stamens	2, creamish, epipetalous, hairy at the base
6.	Length of stamen	20± 0.67mm
7.	Length of filament	19.4±1.05mm
8.	Length of anther	3.4±0.34mm
9.	No. of pollen per flower	59,702
10.	Length of gynoecium	27.6±0.89mm
11.	Stigma	Simple
12.	Style	Slender
13.	Ovary	Superior
14.	Length of the capsule	29.2 ±2.28

Table 2—Values of different floral attributes of *Phlogacanthus thyrsiflorus* determining its absolute and ecological reproductive potentials.

S. No.	Floral Attribute	Value (Mean ± SD)
1	Number of inflorescence per plant	23.8±0.52
2	Number of flowers in an inflorescence	18±0.68
3	Number of fruits per inflorescence	15.8±1.42
4.	Number of seeds per locule	8±0.16

Phlogacanthus thyrsiflorus, the germination percentage was 40.7 % in 100 ppm boric acid with 15 % sucrose solution. It was also observed that both germination percentage and tube length decreases at 25% sucrose solution alone as well as in combination with boric acid (Figs. 1 & 2). This is attributed to the fact that sucrose is necessary for proper pollen nutrition, osmotic control and in combination with boric acid promoted pollen germination. According to Gauch and Duggar (1953) boron combines with sugar to make a sugar-borate complex which is translocated with greater facility rather than non borate sugar molecules. The role of boron has been confirmed in germinating pollen and growing pollen tubes by Sidhu and Malik (1986). Boron may enhance the sucrose uptake and stimulate germinating ability. This observation gets support from Pal *et al.* (1989), Gupta *et al.* (1989), Mandal *et al.* (1982), Bhattacharya *et al.* (1997), Mohi-ud-din *et al.* (2007), and Biswas *et al.* (2008). Several workers also supported the fact that boric acid in combination with sucrose enhances both germination as well as pollen tube development (Mandal *et al.* 1982, Pal *et al.* 1989). Dafni *et al.* (2005) also stated that germination success in sucrose medium might depend on the humidity at which the pollen grains were exposed prior to the germination test and on the age of pollen grains.

Pollen production and morphology-The knowledge of anthesis and pollen production is essential to study of pollination, developing a functional model for forecasting

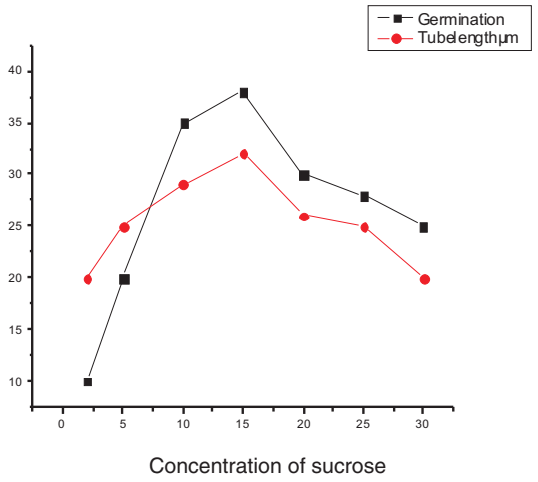

Fig.1–Pollen germination & tube length at different concentrations of sucrose

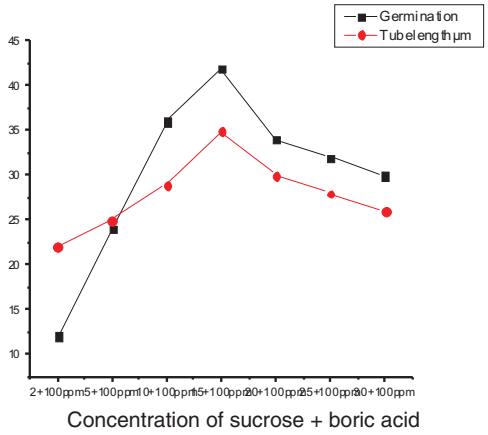

Fig. 2–Pollen germination & tube length at different concentrations of sucrose + boric acid.

pollen concentration and to understand more about the ecological background of pollen dispersal (Davidson 1941). Nair and Kapoor (1974) stated that the studies on pollen production helped to locate the differences in the biological potential of individual flower in an inflorescence. The number of pollen produced per anther varies from 55,690-62,780 (Mean: 59,702). It is evident that pollen production in terms of number per anther varies rather widely from family to family; genus to genus; species to species; even within the different flower of the same plant. The pollen production is a characteristic of all plants, and is by definition, an integral part of the pollination process. The pollen production may affect the percentage of fruit set in plants. Hence it is of great importance in plant improvement programmes. *Phlogacanthus thyrsiflorus* the mode anther dehisces by longitudinal slits.

Pollen grains radially symmetrical, heteropolar, prolate. Polar axis P (37.62-) 33.96±1.25 (-34.52) µm and Equatorial diameter E (24.12-) 25.36±1.47 (-26.12) µm. Pollen grains tricolpate, colpi (29.23–) 30.25±1.79 (-31.42) µm. Exine (1.85 -) 2.17±0.76 (-2.54) µm thick.

Pollen viability—All the dyes used in this experiment for *Phlogacanthus thyrsiflorus s*howed good colour to differentiate between fertile and sterile pollens viz., Muntzing''s mixture (89.20±10.55) and Acetocarmine (82.22±7.50). In the TTC test the percentage of viable pollen is 73.20±6.28 (Plate 3). Nyine and Pillay (2007) also found similar results in their experiments, emphasizing that pollen grain viability assessment through the staining method seem to express the germination potential, but not its occurrence. It may be explained by the fact that this technique overestimates the percentage of pollen tubes formed.

Pollen viability is considered as an important parameter of Pollen quality (Dafni and Firmage 2000). Pollen size and viability are good markers of the course of microsporogenesis. Normal meiosis produces pollen grains regular in size and highly viable, and disturbed meiosis reduces pollen viability and causes variability of pollen grain size (very small and giant pollen are formed in addition to those normal in size); the latter can result from inbreeding depression, autopolyploidy, segmental allopolyploidy, hybridization, mutations, and also environmental effects (Stace 1991).Besides pollen diameter measurement (Kelly *et al.* 2002) the quickest and simplest methods of assessing viability rely on different tests. These results tell us that the term "pollen viability" should be used carefully and rather replaced by the more limited term "pollen stainability," as it depends strictly on the staining assay. A number of authors have discussed the terms used to describe the viability of pollen grains and their ability to germinate and fertilize ovules, and have recommended different terms such as pollen sterility, stainability, viability, geminability, stigmatic geminability, fertilization ability, pollen quality (Dafni and Firmage 2000, Klein 2000). Stainable pollen grains may vary in size and thus be cytologically unbalanced and not viable. Pollen stainability rarely corresponds to pollen germination, which is the best index of pollen viability.

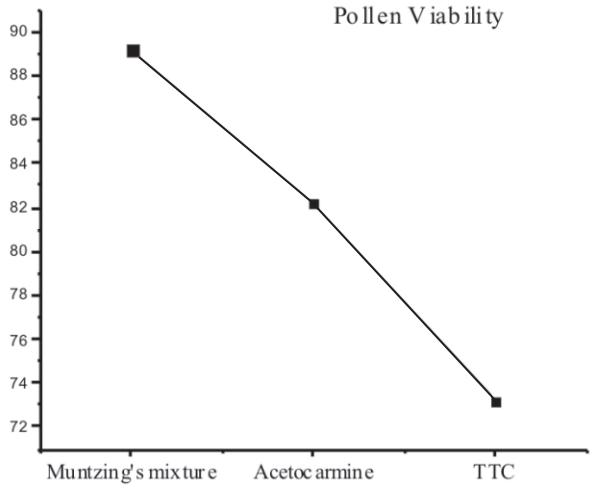

Fig 3–Pollen viability using various dyes.

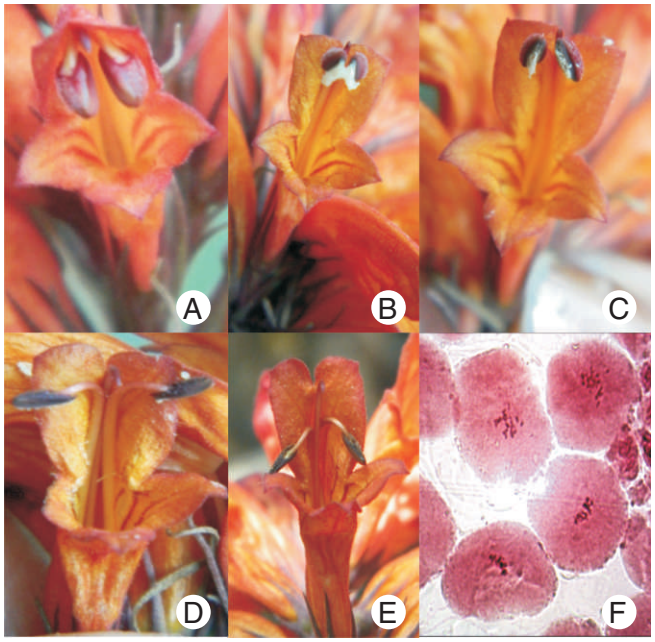

Fig.4— Different stages of pollen biology in *Phlogacanthus thyrsiflorus*. A. 6.00 hr. (flower opening); B. 8.00 hr (anthesis); C. 9.00 hr (anthers start moving); D. 11.00 hr (opposite movement of anthers); E. 13.00 hr (complete bending of anthers) F. Metaphase

Reproductive success—The mean Pollen: Ovule ratio is 59162:1. There was a significant effect of taxonomic position (genus), reward type and pollination mechanism on P/O. Species offering only nectar as a floral reward (which were species with a brush mechanism) had a significantly lower P/O than species offering pollen or pollen and nectar. Species with the brush pollination mechanism had the lowest P/O, while species with valvular and pump mechanism had the highest P/O. However, pollen presentation (primary and secondary) and flower size did not have a significant effect on P/O. The high P/O ratio along with high pollen production in *Phlogacanthus thyrsiflorus* attributes to its high seed set.

Meiotic behaviour–The chromosome number of *Phlogacanthus thyrsiflorus* is 2n=42 (Mehra & Gill 1968a). Different chromosomal stages were observed during meiotic division. The meiosis is normal in the species indicating the normal pollen development (Fig. 4G).

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REFERENCES

Bhattacharya A, Mondal S and Mandal S 1997. *In vitro* pollen germination of *Delonix regia* (Boj.) Raf. *Sci. and Cult.* **63** (5-6) 143-144.

Bhowmik S and Datta BK 2012. *In vitro* pollen germination in *Eichhornia Crassipes* (Mart.) Solms: An insight into its preferred mode of clonal reproduction. *Notulae Scientia Biologicae* **4** (2) 65-71.

Biswas K, Mondal S and Mandal S 2008. Studies on *in vitro* pollen germination of *Solanum surrattense* Burm. f. and *Solanum nigrum* L. *Science and Culture* **74** (3-4) 149 - 152.

Cordell GA 2000. Biodiversity and drug discovery-asymbiotic relationship. *Phytochemistry* **55** 463-480.

Dafni A and Firmage D 2000. Pollen viability and longevity practical, ecological and evolutionary implications. *Plant Systematics and Evolution* **222** 113-132.

Dafni A, Pacni E and Nepi M 2005. Pollen and stigma biology *In*: Dafni A Kevan, PG & Husband, BC (eds.) "*Practical Pollination Biology*" Cambridge, Ontario, Enviroquest 83-142.

Dahanukar SA, Kulkarni RA and Rege NN 2000. Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology* **32** S81-S118.

Davidson R 1941. A note on anthesis in some common grasses near Johannesburg and the relation of anthesis to collection of pollen for medical purposes. *Journal of South African Botany* **7** 145-152.

Davies RR 1969. Climate and topography in relation to aeroallergens at Davos and London. *Acta Allergol* **24** 432-496.

Erdtman G 1952. *Pollen Morphology and Plant Taxonomy-Angiosperms*. Almqvist and Wiksell, Stockholm.

Erdtman G 1960. The acetolysis method – A revised description. *Sv. Bot. Tidsskr.* **54** 261-264.

Faegri K and Iversen J 1964. *Textbook of pollen analysis*. Munksgaard, Copenhagen, 2nd ed.

Gauch Hg and Dugger WM Jr 1953. The role of boron in the translocation of sucrose. *Plant Physiology* **28** 457-466.

Hamilton ED 1959. Studies on the airspora. *Acta allergy* **13** 143-175.

Ji HF, Li XL and Xang HU 2009. Natural products and drug discovery. *EMBO Reports* **10**: 194-200.

Kalita D and Bora RL 2008. Some folk medicines from Lakhimpur district, Assam. *Indian Journal of Traditional Knowledge* **7** (3) 414 - 416.

Kelly JK, Rascha A and Kalisz S 2002. A method to estimate pollen viability from pollen size variation. *Am. J. Bot.* **89** (6) 1021-1023.

Khanikar G 2005. *In*: Sahajlavya Bandarabar Gun. 7th ed. Revised. Guwahati, India, Puthitirtha Prakashan.

Klein M 2000. Pollen studies in plant breeding and selection. *Botanical Guidebooks* **24** 171-193.

Liza SA, Md. Rahman O, Md. Uddin Z, Md. Hassan A and Begum M 2010. Reproductive biology of three medicinal plants. *Bangladesh Journal of Plant Taxonomy* **17** (1) 69-78.

Mandal S and Chanda S 1981. Aeroallergens of West Bengal in the Context of Environmental Pollution and Respiratory Allergy. *Biological Memories* **6** 1-61.

Mandal S, Barui NC and Ganguly PN 1982. *In vitro* pollen germination in *Arachis hypogoea*. *Science and Culture* **48** 115-116.

Mehra PN and Gill LS 1968a. *In*: IOPB chromosome number reports XVI. *Taxon* **17** 199-204

Mohi-ud-din G, Nawchoo IA and Wafai B 2007. Reproductive biology of *Picrorhiza kurrooa* Royle Ex Bnth.-An endangered medicinal plant of the north west Himalaya. *Phytomorphology* **57** 109-116.

Moza MK and Bhatnagar AK 2007. Plant reproductive biology studies crucial for conservation. *Current Science* **92** 243-244. Nair PKK 1964. *Advance Palynology*. National Botanical Garden, Lucknow, India.

Nair PKK and Rastogi K 1963. Pollen production in some allergenic plants. *Cur. Sci.* **32** (12) 566-567.

Nair PKK and Kapoor SK 1974. Pollen production in some vegetable crops. *Geobios* (Jodhpur). **1** 71-73.

Norton JD 1966. Testing of Plum pollen viability with tetrazolium salts. *J. of the Am. Soc. Hort. Sci.* **89** 132-134.

Nyine M and Pillay M 2007. Banana nectar as a medium for testing pollen viability and germination in *Musa*. *African J. Biotech.* **6** 1175-1180.

Pal JK, Datta BK, Mandal S and Bhattacharya GN 1989. Studies on the *in vitro* pollen germination of *Cassia fistula* L. *Mendel* **6** (3-4) 311-315.

Pfahler PL, Pereira MJ and Barnett RD 1997. Genetic variation for *in vitro* sesame pollen germination and tube growth. *Theoretical and Applied Genetics* **95** 1218-1222.

Reis NM and Kostic SR 1976. Pollen season severity and meteorologic parameters in central New Jersy. *The Journal of Allergy and Clinical Immunology* **57** 609-614.

Sedgley M and Griffin AR 1989. *Sexual reproduction of Tree crops*.-Academic Press, London.

Shivanna KR and Johri BM 1985. *The Angiosperm Pollen Structure and function*.Wiley Eastern Ltd. Publisher, New Delhi.

Shivanna KR and Rangaswamy NS 1993. *Pollen Biology – A laboratory manual*. Narosa Publishing House, New Delhi.

Shivanna KR 2003. *Pollen biology and Biotechnology*-Science Publ., Plymouth.

Shrestha BB, Dall'Acqua S, Gewali MB, Jha PK and Innocenti G 2008. Biology and phytochemistry of *Curculigo orchioides* Gaerten. pp. 50-67. *In*: Jha PK, Karmacharya SB, Chettri MK, Thapa CB and Shrestha BB(eds.) *Medicinal Plants of Nepal An Anthology of Contemporary Research.* Ecological Society (ECOS), Kathmandu, Nepal.

Sidhu RJK and Malik CP 1986. Metabolic rate of boron in germinating pollen and growing pollen tubes. *Biotechnology and ecology of pollen* (ed. Mulcahy *et. al.*), Springer, New York 373- 378.

Stace CA 1991. *Plant Taxonomy and Biosystematics* 2nd Ed., Cambridge University Press, Cambridge.

Tandon R, Shivanna KR and Mohan Ram HY 2003. Reproductive biology of *Butea monosperma* (Fabaceae). *Ann. Bot.* **92** 715-723.

Taiz L and Zeiger E 2003. *Plant Physiology* 3rd edn. Panima Publishing Corporation, New Delhi.

Walker JW and Doyle JA 1975. The bases of Angiosperm Phylogeny: palynology. *Ann. Missouri Botanical Garden* **62**(3) 664-723.

Wodehouse RP 1935. *Pollen grains*. McGraw-Hill Book Company, New York.