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Karyo-morphological characterization of natural genetic variation in some threatened *Cymbidium* species of Northeast India

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Abstract — Karyo-morphological studies have been carried out in three different species of the genus *Cymbidium* from North-eastern India. The somatic chromosome number (2n = 40) has been recorded in all the three species with distinct inter-specific variation in the arm ratio of few homologous pairs in the complements. The karyotypes of the species were more or less symmetrical. The significance of karyotypic variation in speciation of the genus *Cymbidium* has been discussed.

Key words: Heteromorphic Chromosome, Karyotype, Mitosis, Orchidaceae, Symmetry.

INTRODUCTION

The data on chromosome number and comparative karyomorphology is very significant to comprehend the genome structure, its organization and evolution within the genus at inter- and intra specific levels (Ehrendorfer 1980). The difference and similarities in the karyotype are regarded as basis of genetic variation, as well as distance or relatedness among diverse genomes. Karyotype studies are regarded as an important cytogenetical tool for elucidating taxonomic and phylogenetic relationships as well as in breeding programme aimed at developing better horticulture types as emphasized by a number of workers (Stace 2000; Singh 1984).

Northeast India is endowed with rich treasure of phyto-diversity including agricultural, horticultural, medicinal and unique floristic plants. It is one of the mega biodiversity hotspot centers (Myers *et al*. 2000) for a number of plant species including various orchids. *Cymbidium*, or boat orchid, is a genus comprising of 52 evergreen species in subtribe Cyrtopodiinae, tribe Cymbidieae and family Orchidaceae. It is distributed in tropical and subtropical Asia (*viz.* North-east India, China, Japan, Malaysia, the Philippines, and Borneo) and North-Australia, usually growing in cooler climates at higher elevations. Raven (1975) reviewed the angiosperm's base number and considered it premature to suggest a distinct base number for Orchidaceae, since chromosome numerical variations in orchids as a whole is quite intriguing and many of the genera exhibit higher ploidy levels with variable base numbers (Goldblatt 1980; Ehrendorfer 1980). Interphase nuclear types and chromosome numbers were observed in 44 species of 20 genera of Cymbidioid orchids occurring in Brazil and the most probable base number for each orchid genus, sub-tribe and tribe of the group has been reported (Felix and Guerra 2000). A good number of workers from China and Japan have tried to investigate the cytogenetical aspects of several orchid species including *Cymbidium* species *viz. C. qiubeiense, C. kanran, C. cyperifolium, C. goeringii, C. serratum, C. longibracteatum and C. faberi* (Li *et al.* 2002a, 2002b, 2003), grown in their native habitat and reported the details on chromosome counts in somatic as well as

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gametic cells, presence of B-chromosomes and aneuploidy/ polyploidy in many of the species investigated. However these studies on orchids are by and large lacking and only a few reports are available on cytological aspects of Indian orchids (SHARMA and CHATTERJI 1961). SINGH (1984) in his research investigations on pollen mitosis observed n = 20 as haploid chromosome number of *C. aloifolium* which was latter observed by several workers in few other species (AOYAMA and TANAKA 1988; FELIX and GUERRA 2000; LONG *et al.* 2000; LI *et al.* 2002a, 2002b, 2003).

Most of the *Cymbidium* plants are epiphytic and hence, root tip mitosis and karyotype analysis is very difficult in the cymbidiums. The basic numbers of quite a good number of other genera belonging to this family is still unclear resulting in difficulties to correctly estimate ploidy levels and to understand the karyological evolution of the family Orchidaceae. Therefore, the present investigations are the first attempt to address such problems dealing with karyotypic studies of three Asiatic *Cymbidium* species *viz. C. eburneum* Lindl., *C. hookerianum* Rchb.f. and *C. mastersii* Griff. ex Lindl., which are threatened in their natural habitat.

MATERIALS AND METHODS

The plant materials used in present investigation were collected from Sikkim region of North-east India. The plants were grown in greenhouse of Plant Biotechnology Laboratory, Department of Botany of North-Eastern Hill University, Shillong. For each species, wherever possible, a minimum of five individuals and more than one population were analyzed. For obtaining actively growing root tips, plants were raised in earthen pots and the root tips of about (0.5-1.0 cm) long were excised and pretreated with saturated solution of ρ-dichlorobenzene for three hours at room temperature followed by fixation in freshly prepared carnoy's fluid for 24 hours. Root tips were hydrolyzed with 5N HCl for 30 minutes at room temperature and stained in 1% leuco-basic fuchsin. The stained tips were squashed in 1% aceto-carmine and the micro-photographs were taken using *Jenoptik* CCD camera (Germany) attached to *Labomed* LX 400 fluorescent microscope. At least five clear preparations of chromosome complements of each species were analyzed for the karyotypes. Idiograms were prepared from photomicrographs by cutting out

TABLE 1 — Means of chromosome length, long/short arm ratio, Karyotypic formula with type of chromosome, and category of symmetry in three species of *Cymbidium*. The value for heteromorphic pairs is underlined.

S.No.	Taxa	2n	r-index in different chromosomes																				Karyo-typic formula	Number of sub-telocentric chromosomes	Ratio of largest/smallest chromosome length	Category of symmetry
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX				
1.	*Cymbidium eburneum*	40	1.05 L	1.36 L	1.18 L	1.00 V	1.18 L	1.33 L	1.00 V	1.20 L	1.07 L	1.81 L	2.00 L	1.32 L	1.56 L	1.58 L	1.30 L	1.10 L	1.47 L	1.28 L	1.20 L	3.00 J	4V+34L+2J	2	2.5	2B
2.	*Cymbidium hookerianum*	40	1.21 L	1.16 L	1.40 L	1.98 L	1.60 L	1.28 L	1.28 L	1.30 L	1.50 L	1.38 L	1.28 L	1.16 L	1.28 L	1.40 L	1.62 L	1.37 L	1.00 V	1.37 L	1.93 L	1.12 L	2V+38L	–	2.05	2B
3.	*Cymbidium mastersii*	40	1.24 L	1.68 L	2.00 L	3.25 J	1.41 L	1.00 V	1.74 L	1.12 L	1.23 L	1.14 L	1.00 V	1.08 L	1.46 L	1.07 L	1.70 L	1.35 L	1.50 L	2.00 L	2.00 L	3.00 J	4V+32L+4J	4	2.37	2B

individual chromosomes, arranging them in descending order of their length and matching on the basis of morphology. The standard method of chromosome classification (Battaglia, 1955) of median (V), submedian (L), subtelocentric (J) and telocentric (I) based on the arm ratio of 1:1, >1:1<1:3, >1:3<1:0 and 1:0 respectively, was used for comparison. The degree of symmetry was estimated as per the scheme proposed by Stebbins (1971).

RESULTS

Mitotic data on three species of cymbidiums i.e *C. eburneum*, *C. hookerianum* and *C. mastersii* are summarized in Table 1 and illustrated in Fig. 1-9.

Chromosome complements - All the three species had shown the occurrence of 2n = 40 chromosomes in root tip cells which were clearly resolved into 20 pairs forming a series from the longest to shortest pair within the complements. In *C. eburneum* two metacentric and seventeen submetacentric pairs were observed while only one pair was found to be subtelocentric in nature. The longest chromosome in the complement was two and half times longer than the smallest one. The first pair was found to be heteromorphic in nature. Karyotypic formula was resolved into 4V + 34L + 2J (Table 1). However in *C. hookerianum,* only one pair was metacentric and rest of the nineteen pairs were found to be submetacentric in nature. The longest chromosome was almost two times longer than the smallest one. No heteromorphic chromosomes were recorded. Karyotypic formula was resolved into 2V + 38L (Table 1). On the other hand in *C. mastersii* two metacentric and sixteen submetacentric chromosome pairs were recorded while the remaining two pairs were subtelocentric in nature. The longest chromosome was more than two times longer than the smallest one. Sixteenth pair of the chromosomes was found to be heteromorphic in nature. Karyotypic formula in this species has been resolved into 4V + 32L + 4J (Table 1). One notable feature of the present observation was lack of distinct nucleolar chromosomes in the complements in any of the three species analyzed.

Variation was recorded with respect to number of metacentric and submetacentric chromosomes, presence or absence of heteromorphic pairs in the chromosome complements of *C. eburneum, C. hookerianum* and *C. mastersii*. In the present investigation, the plants belonging to *C. hookerianum*, are characterized by the presence of either metacentric or submetacentric chromosomes with no subtelocentric pair in karyotype. On the other hand, *C. eburneum* and *C. mastersii* are distinct with a minimum of one pair of subtelocentric chromosomes in the complement.

The chromosome morphology with regard to a particular pair in the karyotype has shown significant variation at inter-specific level (Table 1). For example, the fourth pair in C. *eburneum* is metacentric where as in C. *hookerianum* it is submetacentric while in *C. mastersii*, it is found to be subtelocentric in nature. Such observation can be extended even too few other pairs (i.e. VI, VII, XI, XVII, and XX) as well. In *C. eburneum* and *C. mastersii*, the first and sixteenth pair of the chromosomes was recorded as heteromorphic pairs respectively (Fig. 3 and 9), which served as cytogenetical marker. Alternatively, not a single pair of the chromosome was found to be heteromorphic in nature in case of *C. hookerianum* (Fig. 6).

Symmetry - Following the classification of STEBBINS (1971), the karyotypes in all the three species of the genus *Cymbidium* studied were resolved in to 2B category. (Table1)

DISCUSSION

Cytological data on Indian orchid flora is available for only a few genera and most of them are restricted to chromosome counts only (ARORA 1960; SHARMA and CHATTERJI 1961; MEHRA and YASHPAL 1961; MEHRA and BAWA 1962; CHENNAVEERAIAH and JORAPUR 1966). SHARMA and CHATTERJI (1966), from their investigations on 35 species of orchids belonging to 17 genera, observed the presence of a wide spectrum of basic numbers within each tribe and genus of family Orchidaceae. The genus *Cymbidium* did attract a number of biologists from time to time to study a range of research aspects such as morphology (ATWOOD 1984); taxonomy (ALBERT and PETTERSSON 1994); pollination biology (CHRISTENSEN 1994) and in recent time molecular systematics (COX *et al.* 1997); molecular marker based phylogeny (OKEYO and KAKO 1998; VAN DEN BERG *et al.* 2000, 2002; XIAOHONG *et al.* 2007) and *in-vitro* reproduction studies (JAIME *et al.* 2006). However, from cytogenetical and karyological point of view, few reports are available. SINGH (1984) in his reports on pollen mitosis of

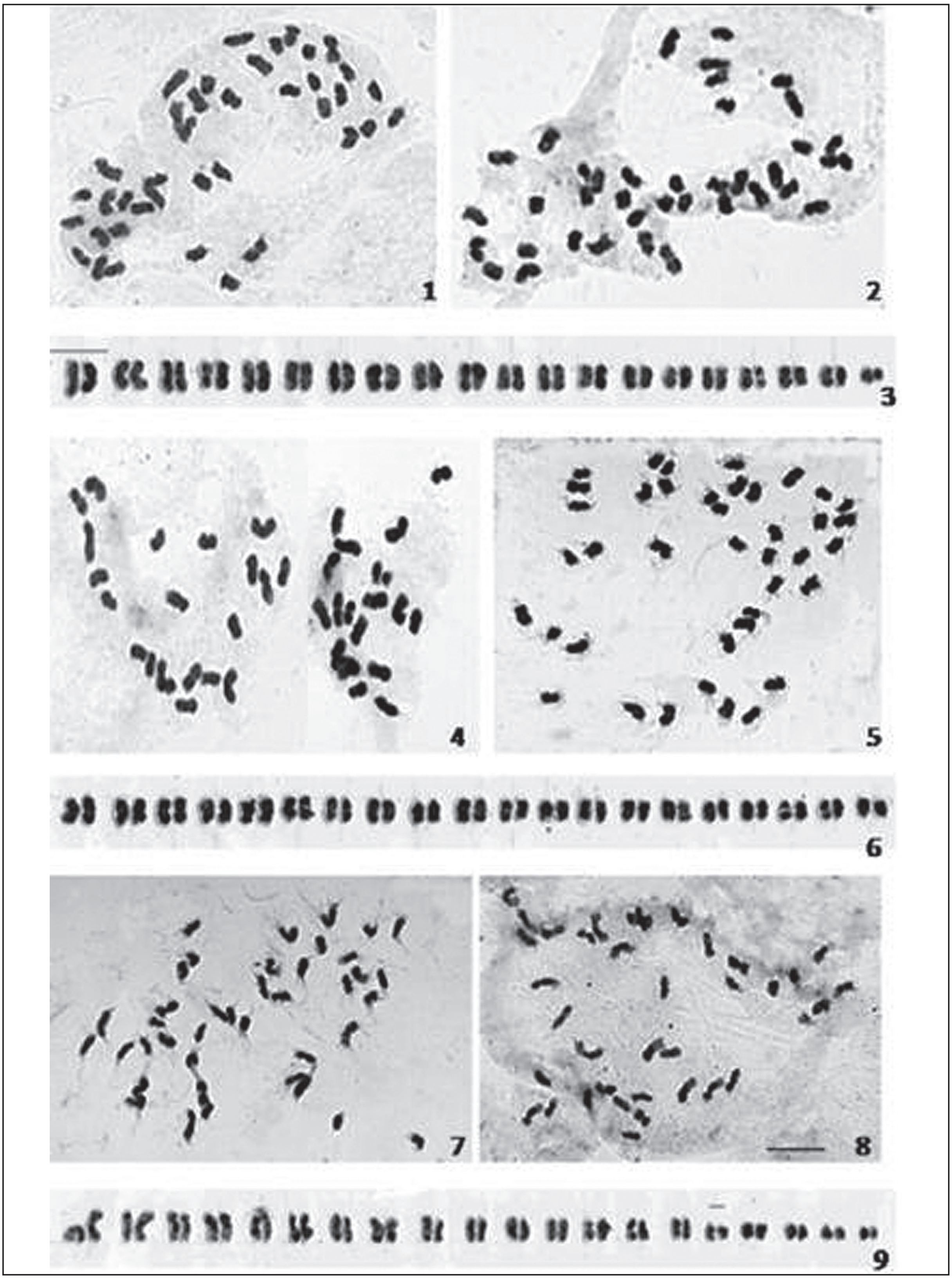

Figure 1-9 — Mitotic complements of *Cymbidium* species. (1-2) *C. eburneum*, 2n = 40; (3) photo-idiogram of *C. eburneum*. (4-5) *C. hookerianum*, 2n = 40; (6) photo-idiogram of *C. hookerianum*. (7-8) *C. mastersii*, 2n = 40; (9) photo-idiogram of *C. mastersii*. Bar = 10μm. Heteromorphic pair marked above the short arm.

Cymbidium aloifolium reported haploid chromosome number of n = 20, thereby suggesting somatic chromosome number of 2n = 40. Karyological studies in various species and varieties of *Cymbidium* from China (Li *et al.* 2002a; 2002b; 2003) and Japan (Aoyama 1989) are also on record. Except these findings the genus *Cymbidium* did not find favor with any cytogeneticists perhaps owing to rarity of the plants in nature and difficulties in maintaining them under cultivation. Thus, the present investigation is first attempt to record karyo-morphological details on *C. eburneum*, *C. hookerianum* and *C. mastersii.*

In the present study, characteristic differences have been recorded in karyotypes at inter-specific level of the genus *Cymbidium.* In general, fourteen pairs out of twenty *viz.* I-III, V, VII-X, XII-XVI and XVIII-XIX, shows uniformity with respect to the chromosome morphology at inter-specific level indicating the high degree of genomic stability in the genus. However, moderate to greater degree of variation was recorded in the remaining six pairs of the chromosome complements pattern, such variation with respect to V, L, J types of chromosomes may result due to structural changes in chromosomes *viz.,* duplication, deletions, interchanges and inversions (Rao and Chandel 1991; Stebbins 1971).

Felix and Guerra (2000) have published some excellent cytogenetical treatises on orchids especially on members of Cymbidioid phylad. About 44 species belonging to Cymbidioid genera were cytogenetically characterized and pattern of karyological evolution within the group was reported. The chromosome variability reported by them ranges from 2n = 10 (*Psygmorchis pusilla*) to 2n = 168 (*Oncidium* species). They have also investigated various sub-tribes for chromosome counts and recorded variation both within and between sub-tribes, which was quite remarkable. They were of the opinion that orchids in general and Cymbidioid phylad in particular have extensively benefited by the occurrence of variable base numbers followed by attainment of higher ploidy levels. The present authors are in complete agreement with such observation of Felix and Guerra (2000).

From the review of earlier published chromosome counts of *Cymbidium* and allied species from various parts of the world viz. Brazil (Felix and Guerra 2000), China (Li *et al.* 2002a; 2002b; 2003) and Japan (Aoyama and Tanaka 1988; Aoyama 1989), it can be observed that barring few exceptions, the genus *Cymbidium* has adopted x = 10 as the basic number and therefore majority species reveled somatic chromosome number 2n = 40. The present investigation on three species of *Cymbidium* viz. *C. eburneum*, *C. hookerianum* and *C. mastersii* also record the same tendency with regard to x = 10 as true basic number of the genus *Cymbidium*. However, certain deviant chromosome counts of 2n = 38, 42 and 52 in species like *C. eburneum*, *C. bicolor* and *C. iridiodes* collected from Asian region by some authors (Aoyama and Tanaka 1988; Aoyama 1989) need to be further investigated vis-à-vis the proposed basic number of x = 10 for the geuns *Cymbidium*.

The genus *Cymbidium* is known for consistency in somatic chromosome numbers (2n = 40) and the lone exception of 2n = 38 in subgenus *Jensoa* has been reported by Aoyoma and Tanaka (1988). Besides these unique observations on chromosome counts, they have also reported the occurrence of significant number of B- chromosomes in various *Cymbidium* species, whose number ranged from 1-5 in *C. lancifolium* and *C. javanicum.* Occasional occurrence of triploid cytotypes was yet another novel finding recorded by Aoyoma and Tanaka (1988). Despite such convincing and compelling observation on B- chromosomes and aneuploidy, in the present investigation, the authors did not come across any such deviations in any of the materials investigated from North-east Indian region.

While assessing the pattern of chromosome morphology and karyotypes Li *et al.* (2002b) have observed symmetrical karyotypes (2B) in seven species and one verity of the genus *Cymbidium* from China. In the present study, the ratio of longest and shortest chromosome had exceeded more than 2.5. Sub-telocentric/telocentric chromosome pair (1 number) was recorded in *C. eburneum* and two in *C. mastersii* as well. Absence of any deviant chromosome numbers and numerical/ structural changes in chromosomes suggest more or less stabilized genome of the genus *Cymbidium.* The present authors also derive support for such observations in the form of 2B level of symmetry in the three species presently investigated. This is also an indication of possible stability, which the *Cymbidium* species attained in respect of chromosome structure and morphology.

The observation related to heteromorphic pairs recorded in *C. eburneum* and *C. mastersii* is indicative of the fact that these pairs in the chromosome complements comparatively exhibit less genome integrity and thereby helping the species to attempt in structural alterations as means of speciation. The absence of any deviant chromosome numbers other than x = 10 and overall symmetry suggests that the diversification at inter-specific level has occurred through structural alteration of chromosome rather than numerical change. Not a single pair of nucleolar organizers observed in the form of secondary constriction in any of the three species may be attributed to technical difficulties. In such a situation the modern cytogenetical tools *i.e.,* in situ hybridization *viz.,* fluorescent in situ hybridization (FISH) and multicolour fluorescent in situ hybridization (McFISH) may be of great help to characterize the ribosomal DNA loci and can also resolve the problems providing an insight in to heteromorphic chromosomes based speciation in the genus *Cymbidium*.

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REFERENCES

Albert V.A., and Pettersson B., 1994 — *Expansion of genus paphiopedilum to include all conduplicate-leaved slipper orchids (Cypripedioideae: Orchidaceae)*. Lindleyana, 9: 133-139.

Aoyama M., and Tanaka R., 1988 — *Notable chromosome numbers in Cymbidium lancifolium, C. javanicum and C. nipponicum.* J. Jap. Bot., 63: 329-333.

Aoyama, M., 1989 — *Karyomorphological studies in Cymbidium and its allied genera*. Bulletin of Hiroshima Botanical Garden, 11: 1-121.

Arora C.M., 1960 — *New Chromosome report. Bulletin of Botanical Survey of India*, 2: 305.

Atwood J.T.(Jr.)., 1984 — *The relationships of slipper orchids (sub-family Cypripedioideae: Orchidaceae)*. Selbyana, 7: 129-147.

Battaglia E., 1955 — *Chromosome morphology and terminology*. Caryologia, 6: 179-187.

Chennaveeraiah M.S. and Jorapur S.M., 1966 — *Chromosome Number and morphology in five species of Nervilia Gaud*. Nucleus, 9: 39-44.

Christensen D.E., 1994 — *Fly pollination of Orchidaceae*. In: J.Arditti (ed.): Orchid Biology: reviews and perspectives. John Wiley and Sons, Newyork. VI: p. 415-454.

Cox A.V., Pridgeom A.M., Albert V.A. and Chase M.W., 1997 — *Phylogenetics of slipper orchids (Cypripedioideae: Orchidaceae): nuclear rDNA sequence*. Plant Systematics and Evolution, 208: 197-223.

Ehrendorfer F., 1980 — *Polyploidy and distribution*. In: Lewis, W. H. (ed.): Polyploidy: Biological relevance Plenum Press, New York, p. 45-60.

Felix L.P. and Guerra, M., 2000 — *Cytogenetics and cytotaxonomy of some Brazilian species of Cymbidioid orchids*. Genetics and Molecular Biology, 23: 957-778.

Goldblatt P., 1980 — *Polyploidy in angiosperms: monocotyledons*. In: Lewis, W.H. (ed.): Polyploidy : Biological relevance. Plenum Press, New York, p. 219-232.

Huang B.Q., Ye X.L., Yeung E.C. and Zee S.Y., 1998 — *Embryology of Cymbidium sinense: the microtubule organization of early embryos*. Annals of Botany, 81: 741-750.

Jaime A., Da silva T., Singh, N. and Tanaka M., 2006 — *Priming biotic factor for optimal protocorm-like body and callus induction in hybrid Cymbidium (Orchidaceae), and assessment of cytogenetic stability in regenerated plantlets*. Plant Cell, Tissue and Organ culture, 84: 135-144.

Li Y.G., Guo W. H., and Wu B. J., 2002a — *Studies on karyotypes of four species of Cymbidium in China.* Acta Bot. Bor. Occid. Sin. 22: 1438-1444.

Li Y.G., Guo W. H., and Wu B. J., 2002b — *A karyological study of seven species and one variety of Cymbidium from China.* Acta Phytotaxonomica Sinica, 40: 406-413.

Li Y.G., Guo W. H., and Wu B. J., 2003 — *A karyological study of six Chinese species of Cymbidium.* Acta Bot. Yunnanica 25: 83-89.

Long Y.Q., Fu H.L., Su L.J., 2000 — *A study on karyotype of Cymbidium goeringii (Rchb.f.)* J. Sichuan Univ. Nat. Sci. Ed. 37: 578-581.

Mehra P.N. and Bawa K.S., 1962 — *Chromosome studies in Orchidaceae*. Proc. 49th Indian Science Congress. Part III, p. 326-327.

Mehra P.N. and Yashpal., 1961 — *Cytological observation on some Indian members of orchidaceae*. Proc. 48th Indian Science Congress, part III, p. 294.

Myers N., Mittermeier R.A., Mittermeier C.G., Fonseca G.A.B. and Kent J. 2000 — *Biodiversity hotspots for conservation priorities*. Nature, 403: 853-858.

Obara-Okeyo P. and Kako S., 1998 — *Genetic diversity and identification of Cymbidium cultivars as measured by random amplified polymorphic DNA (RAPD) markers*. Euphytica, 99: 95-101.

Rao S.R. and Chandel K.P.S., 1991 — *karyomorphological studies in cultivated Vigna species in Indian gene center*. Cytologia, 56: 47-57.

Raven P.H., 1975 — *The bases of angiosperm phylogeny: cytology*. Ann. MO Bot. Gard, 62:724-764.

Sharma A. K. and Chetterji A.K., 1966 — *The chromosome numbers of few more orchid genera*. Current Science, 30: 75.

Singh F., 1984 — *Pollen mitosis studies in orchids with particular reference to Cymbidium aloifolium SW.* Current Science, 53: 988-992.

STACE C.A., 2000 — *Cytology and cytogenetics as a fundamental taxonomic resource for the twenty and twenty first centuries*. Taxon, 49: 451-477.
STEBBINS G. L., 1971 — *Chromosomal Evolution in Higher Plants*. Edward Arnold, London.
VAN DEN BERG C., HIGGINS W.E., DRESSLER R.L., WHITTEN W.M., SOTO ARENAS M.A., CULHAM A. and CHASE M.W., 2000 — *A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from the internal transcribed spacers (ITS) of nuclear ribosomal DNA*. Lindleyana, 15:96-114.
VAN DEN BERG C., RYAN A., CRIBB P.J. and CHASE M.W., 2002 — *Molecular phylogenectics of cymbidium (Orchidaceae: Maxillarieae): Sequence data from the internal transcribed spacers (ITS) of nuclear ribosomal DNA and plastid matK*. Lindleyana, 17: 102-111.
XIAOHONG Y., LI G. and BO Y., 2007 — *Genetic diversity of wild Cymbidium goeringii (Orchidaceae) populations from Hubei based on Inter-simple sequence repeats analysis.* Front. Biol. China., 2: 419-424.

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