CARYOLOGIA Vol. 54, no. 2: 121-127, 2001

Karyomorphology of *Podocarpus* s.l. in China and its systematic significance

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Abstract – Investigated in the present paper is the karyomorphology of 7 species in 3 sections of *Podocarpus* s.l. from Yunnan province of China. All species commonly showed the mitotic prophase chromosomes of the same interstitial type, but different resting nuclei type. The resting nuclei were found the gradient type for Sect. Dacrycarpus, the simple chromocenter type for Sect. Nageia and the complex chromocenter type for Sect. Podocarpus respectively. The karyotype formulae were $2n$=20=16M+4SM for *Podocarpus imbricatus*, $2n$=26=9M+17SM for *P. nagi*, $2n$=26=17M+9SM for *P. wallichiana*, $2n$=26=13M+13SM for *P. fleuryi*, $2n$=38=19M+9SM+2ST+8T for *P. macrophyllus* var. *angustifolius*, $2n$=38=18M+10SM+2SM+8T for *P. forestii*, and $2n$=38=20M+10SM+8T for *P. neriifolius* respectively. The karmorphology of 3 sections in *Podocarpus,* especially the resting nuclei and basic chromosome number, together with the other data, justified their generic status in the family.

Key words: karyology; *Podocarpus*; systematics.

INTRODUCTION

The subfamily (or tribe) Podocarpeae was established by ENDLICHER (1847), including 3 genera, the type genus *Podocarpus*, *Dacrydium* and *Microcachrys*. And 4 sections were described in *Podocarpus*, i.e. Sect. Nageia, Sect. Eupodocarpus, Sect. Stachycarpus and Sect. Dacrycarpus. But in PILGER's system (1926), the 7 genera, *Podocarpus, Dacrydium, Phyllocladus, Microcachrys, Pherosphaera, Saxegothaea* and *Acmophyle* within the family Podocarpaceae were considered, and 7 families including the Podocarpaceae were described in conifers. Also in his system, *Podocarpus* included two subgenera, Subgenus I: Sta[illegible] and Subgenus II: Protopodocarpus, while 4 sections were included in Subgenus II. PILGER's (1926) treatment of the Podocarpaceae [illegible] of scholars such as FLORIN (1931, [illegible] (1953), CHENG and FU (1978) and [illegible]

But, after his research on the morphology and anatomy, DE LAUBENFELS (1969) found that differences between the sections within *Podocarpus* were so large as between genera. Combined with his revision of *Nageia* (1987), the sections in *Podocarpus* were upgraded as different genera. 13 genera including *Podocarpus*, *Nageia*, *Dacrycarpus* were founded in the Podocarpaceae. Followed DE LAUBENFELS, PAGE (1988, 1990) put forward a new system, which 17 genera were justified in the Podocarpaceae. Along with the increasing number of genera in the family, some genera were separated as different monotypic families. For example, *Phyllocladus* was considered as Phyllocladaceae by KENG (1973, 1979) after his series studies; *Nageia* was upgraded as Nageiaceae by FU (1992) for the special multiveined leaves and reproductive organ in the genus. Thus it can be seen; the systematic position of different sections in *Podocarpus* was still confused.

Only 3 sections of *Podocarpus* s.l. can be found in China, 1 species in Sect. Dacrycarpus, 4 species in Sect. Nageia and 9 species in Sect. Podocarpus according to CHENG and FU's system (1978). The basic chromosome number of the

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Podocarpaceae is variable largely (HAIR and BEUZENBERG 1958), and they can present valuable suggestions to the relationships and systematic implications of these taxa in the *Podocarpus* s.l. In this paper, we intended to discuss the systematic position of 3 Chinese sections of the genus from cytological investigation.

MATERIALS AND METHODS

7 taxa in 3 sections of *Podocarpus* were collected from Xishuangbanna of Yunnan except *P. imbricatus* from Pingbian of Yunnan, *P. forestii* from Dali of Yunnan province in China. All were cultivated in the Kunming Botanic Garden of Kunming Institute of Botany, the Chinese Academy of Sciences. Voucher specimens are deposited in the herbarium of the institute (KUN). The 7 taxa were *P. imbricatus* Bl. (Voucher No.: Zhou Qixing 200) of Sect. Dacrycarpus, *P. nagi* (Thunb.) Zoll. et Mor. ex Zoll. (Gu Zhijian 9731902), *P. wallichiana* Presl (Gu Zhijian 9731903) and *P. fleuryi* Hickel (Gu Zhijian 9731908) of Sect. Nageia, *P. macrophylla* (Thunb.) D. Don var. *angustifolius* Bl. (Gu Zhijian 9731906), *P. forrestii* Craib et Smith (Zhou Qixing 202), *P. neriifolius* D. Don (Gu Zhijian 9731904) of Sect. Podocarpus.

The roots of female individuals of all taxa were harvested and pretreated with 0.1% colchicine for 8 hours, and then fixed for 30 minutes in Carnoy's fluid (absolute alcohol: glacial acetic acid=3:1) at about 0°C. After being macerated in a mixture of 1 mol/L hydrochloric acid and 45% glacial acetic acid (1:1) at 60°C for two minutes, they were stained and squashed with 1% aceto-orcein. The cytological classifications of the resting and mitotic prophase followed TANAKA's category (1971, 1977). The symbols for the description of chromosomes followed LEVAN *et al.* (1964). The classification of karyotype asymmetry was estimated according to STEBBINS (1971).

RESULTS

The present paper reported the cytology of 7 species in three sections of *Podocarpus* s.l. in China. The karyotype structure is shown in Table 1.

1. Sect. Dacrycarpus

Of the section, only *Podocarpus imbricatus* is found in China. The resting nucleus (Fig 1A) belongs to gradient type, characterized by one large darkly staining block at one side of cell. In the mitotic-prophase stage, heterochromatic and euchromatic segments were distinguishable, and the darkly stained heterochromatic dots were found in the distal chromosome ends. Therefore, the prophase chromosomes are classified as the interstitial type (Fig. 1B).

The karyotype is formulated as $2n$=20=16M +4SM. The basic chromosome number was X=10. Secondary constrictions were found in the long arms of the 1st, 2nd, 7th, 12th, 17th, 19th, and in the short arm of the 5th, 6th, 13th chromosomes (Fig. 1C; Fig. 2A). The ratio of the longest to the shortest chromosome was 1.38 and only 5.0% chromosomes' arm ratio was over 2.00. Asymmetry of the karyotype is categorized to be 1A type.

2. Sect. Nageia

3 species of the section were investigated. Their resting nuclei (Fig. 1D) belong to the simple chromocenter type, characterized by many darkly staining and rough surfaced heteropycnotic bodies. The mitotic-prophase chromosomes (Fig. 1E) belonge to the interstitial type, similar to those of Sect. *Dacrycarpus*. The chromosome number of 3 species in the present study is found

Table 1 – Karyotypic structure of 7 taxa in *Podocarpus*.

Taxa	Chromosome number ($2n$)	Relative length	Karyotype structure M	SM	ST	T	Type
Sect. Dacrycarpus							
Podocarpus imbricatus	20	4.26~5.86	16	4	–	–	1A
Sect. Nageia							
P.nagi	26	2.48~5.55	9	17	–	–	2B
P.wallichiana	26	2.81~5.02	17	9	–	–	2A
P.fleuryi	26	2.62~5.31	13	13	–	–	2B
Sect. Podocarpus							
P. macrophylla var. *angustifolius*	38	1.01~4.59	19	9	2	8	2C
P. forrestii	38	1.50~4.55	18	10	2	8	2B
P. neriifolius	38	1.34~4.45	20	10	–	8	2B

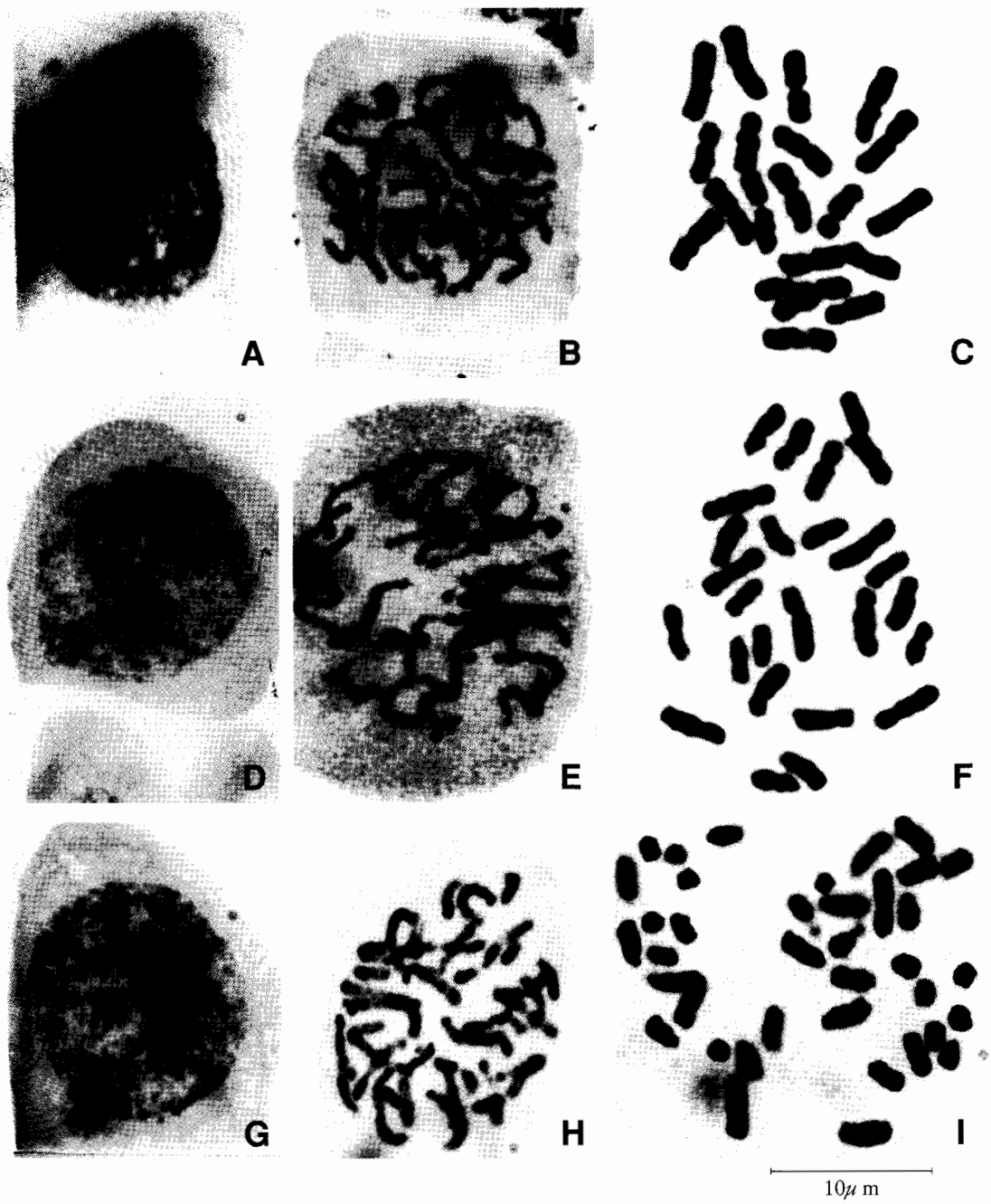

Fig. 1 A, D, G – The resting nucleus of Sect. Dacrycarpus, Sect. Nagiea and Sect. Podocarpus respectively; B, E, H – The prophase chromosomes of Sect. Dacrycarpus, Sect. Nagiea and Sect. Podocarpus respectively; C, F, I – The metaphase chromosomes of Sect. Dacrycarpus, Sect. Nagiea and Sect. Podocarpus respectively.

to be $2n$=26, with basic chromosome number X=13.

Podocarpus nagi (Fig. 2B)

The metaphase chromosomes of *P. nagi* comprise 9 m- and 17 sm-chromosomes. And karyotype is formulated as $2n$=26=9M+17SM. The ratio of longest to the shortest chromosome was 2.24, while 30.8% of the chromosomes' arm ratio is over 2.00. Secondary constrictions are found in the long arms of the 6th, 9th chromosomes, and 1 satellite always exists at the tips of long arm of 3rd and 4th chromosomes respectively. Asymmetry of the karyotype is categorized to be 2B type.

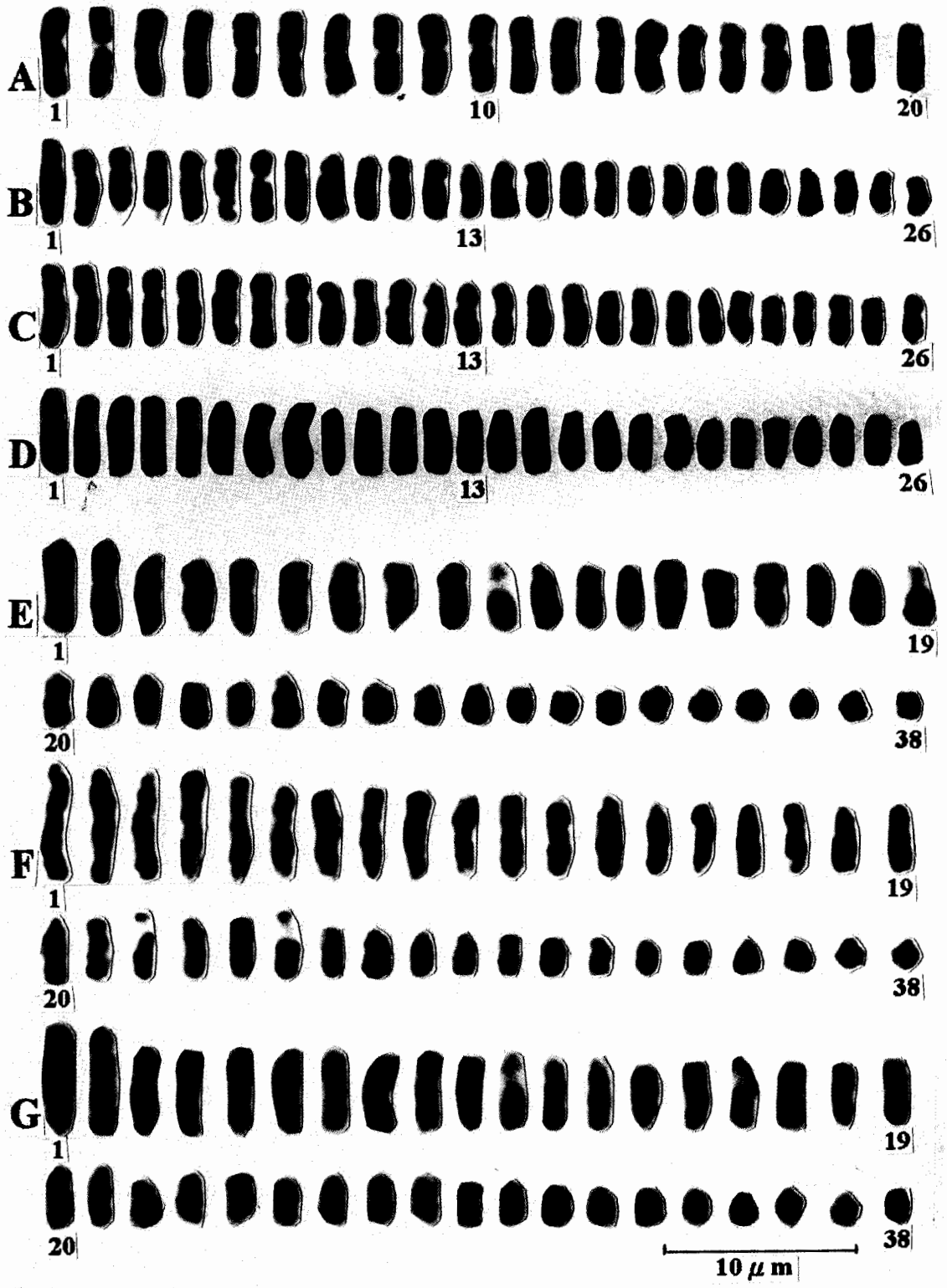

Fig. 2 – Karyograms of 7 taxa in 3 sections of *Podocarpus*. A. *Podoarpus imbricatus*, B. *P. nagi*, C. *P. wallichiana*, D. *P. fleu*
E. *P. macrophyllus* var. *angustifolius*, F. *P. forrestii*, G. *P. neriifolius*.

Podocarpus wallichiana (Fig. 2C)

17 m- and 9 sm-chromosomes are found in the somatic cells of *P. wallichiana*. The karyotype of the species was formulated as $2n$=26=17M+9SM. The ratio of longest to shortest chromosome is 3.1% of the chromosomes whose arm 2.00 can be measured. Secondary constrictions are found in the long arms of the 3rd th, 6th chromosomes. Asymmetry of the karyotype is categorized to be 2A type.

Podocarpus fleuryi (Fig. 2D)

The karyotype is formulated as $2n$=26=13M+13SM. The ratio of longest to the shortest chromosome is 2.03, the arm ratio of 26.9% of the chromosomes is over 2.00. Secondary constrictions are found in the long arms of the 4th, 7th, 9th, 10th chromosomes. Asymmetry of the karyotype is categorized to be 2B type.

Sect. Podocarpus

In this section, 3 representative species are investigated. Their resting nuclei (Fig. 1G) are of the complex chromocenter type, characterized by many darkly staining and random arranging heteropycnotic blocks, which vary in size and number. In the mitotic-prophase stage, the prophase chromosomes (Fig. 1H) belong to the interstitial type, similar to the Sect. Dacrycarpus and Sect. Nageia. 38 metaphase chromosomes are counted in Sect. Podocarpus. And basic chromosome number x=19 is suggested.

Podocarpus macrophyllus var. *angustifolius* (Fig. 2E)

The karyotype are formulated as $2n$=38=19M+9SM+2ST+8T. The ratio of the longest to the shortest chromosome was 3.40, and 21.1% of all the chromosomes' arm ratio was over 2.00. And satellites were found in the 19th, 25th chromosomes. Asymmetry of the karyotype is categorized to be 2C type.

Podocarpus forrestii (Fig. 2F)

The metaphase karyotype is formulated as $2n$=38=18M+10SM+2ST+8T. The ratio of the to the shortest chromosome is 4.54, the chromosomes is also found that the arm ratio over 2.00. Asymmetry of the karyotype is categorized to be 2B type.

Podocarpus neriifolius (Fig. 2G)

No st-chromosme is measured in this species. And karyotype is formulated as $2n$=38=20M+10SM+8T. The ratio of the longest to the shortest chromosome is 3.03. Only 18.4% of all the chromosomes is measured which arm ratio over 2.00. Asymmetry of the karyotype is categorized to be 2B type.

DISCUSSION

The prophase chromosomes of all the 3 sections are of the same interstitial type. As most taxa of gymnosperms own the same prophase chromosome type, they can't get enough implications based on the characters of prophase chromosomes. But the resting nuclei and the chromosome basic number were quite different from each other. And the characters of resting nuclei were relatively stable within genus, they were valuable to the discussion on the systematic positions. Different resting nuclei types implied the systematic and evolution significances at the genera or even higher hierarchy according to TANAKA's studies on the Orchidaceae (1977). Generally, the basic chromosome numbers were mostly stable within genus in the gymnosperms. The metaphase chromosome number counted to be $2n$=26 for Sect. Nageia in the present paper was similar to those early reports (ZOU 1982; HUANG *et al.* 1989; CHEN and HUANG 1989). The diploid with basic chromosome number X=13 can be suggested. And we found the basic chromosome number of Sect. Podocarpus in the present paper was also stable within section, although early reports found that chromosome number of *Podocarpus* were variable (FLORY 1936; MEHRA and KHOSHOO 1956; HAIR and BEUZENBERG 1958; HIZUME *et al.* 1988). Thus, basic chromosome number x=19 for Sect. Podocarpus can be supported.

Besides, the chromosome variations in size were found valuable too. The metaphase chromosomes in the Sect. Dacrycarpus varied very gently in size, and the ratio of the longest to the shortest chromosome was 1.38. While in the Sect. Podocarpus, the chromosomes sharply changed in size, with the ratios of the longest to the shortest chromosome up to 3.03 ~ 4.54. And the variation degree in chromosome size of Sect. Nageia was 1.79 ~ 2.24, just between Sect. Dacrycarpus and Sect. Podocarpus. Inferred from the different resting nuclei type, basic chromosome number and the chromosome size variation, it is,

therefore, inapposite to put 3 sections of *Podocarpus* together.

Obviously, we can easily distinguish the 3 Chinese sections of *Podocarpus* from external morphology. The leaves of Sect. Dacrycarpus were small and spirally arranged. One obvious midvein and one resin duct, stoma lines on both epidermis were also observable. But the leaves of all the species in Sect. Nageia were broadly ovate-elliptic to oblong-lanceolate with many parallel lengthwise veins but without obvious midvein. The corresponding resin ducts were also found at the abaxial side of most vascular bundles. Unlike the above two sections, the spirally arranged or subopposite leaves of Sect. Podocarpus were mostly linear-lanceolate with one midvein. 3 resin ducts can be observed under the vascular bundle.

As introduced in the forward of this paper, different taxonomists variously treated the systematic position of 3 Chinese sections of *Podocarpus* for a long period. Some taxonomists considered these taxa as sections in the *Podocarpus*. But some other taxonomists separated them as different genera in the Podocapaceae. Even some genera were upgraded as monotypic families, i.e. KENG (1973) treated *Phyllocladus* as Phyllocladaceae and FU (1992) treated *Nageia* as Nageiaceae.

Based on the sequences of 18S rRNA, CHAW *et al.* (1995, 1997) found that genus *Nageia* was most similar to the *Podocarpus* and *Dacrycarpus*. And they considered *Phyllocladus* and *Nageia* should better be the member of the Podocarpaceae rather than separate families. WANG and SHU (2000) also support the close relationship between *Nageia* and *Podocarpus* inferred from chloroplast *matK* gene sequences. In the revision of the Podocarpaceae, FU *et al.* (1999) upgraded the 3 Chinese sections of *Podocarpus* as different genera.

Formerly, we have also compared the leaf anatomy and morphology of Chinese Podocarpaceae, found that the characters were coincidence within genus, but different between genera. The 3 sections of *Podocarpus* s.l. were found different in the number of resin ducts and vascular bundles, the arrangements of resin ducts and with or without midvein (in press). With the molecular evidences (CHAW *et al.* 1995, 1997; WANG and SHU 2000) and morphology of reproductive organ (CHENG and FU 1978; DE LAUBENFELS 1969, 1987; FU *et al.* 1999), we suggested the 3 Chinese sections of *Podocarpus* s.l. should be treated as different genera or even higher hierarchy.

Acknowledgements – This work was financially supported by the National Natural Science Foundation of China (39870089); the Pilot Project of the State Knowledge Innovation Programme of the Chinese Academy of Sciences (CAS). Also, valuable comments from the anonymous reviewers are acknowledged.

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Received January 10, 2001; accepted March 7, 2001