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A Contribution to the Pollen Morphology of Hippocastanaceae*[1]

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칠엽수科 花粉의 形態學的 硏究*[1]

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ABSTRACT

The pollen morphology of 20 species and one variety of the two genera of Hippocastanaceae was investigated by LM, SEM and TEM. The pollen grains of this family are monads, prolate to subprolate, isopolar, radially symmetrical, 3-colporate or 3-colpate and ora is circular or lolongate. Sculpture pattern is distinctly striate or spinulose, lirae in various directions. Based on the aperture types and sculpture patterns three major pollen types of this family are discernible : *hippocastanum*, *indica*, and *punduana*.

Key words : Aesculus, *Pollen key*.

요 약

칠엽수科 2屬의 20種 1變種에 대한 화분 형태를 광학현미경, 주사형전자현미경 그리고 투과형전자현미경을 이용하여 관찰하였다. 화분립은 단립, 장구형 내지 아장구형, 등극성, 방사성 대칭, 발아구는 3공구형 혹은 3구형이며 내공구는 원형 또는 횡장형이다. 표면무늬는 유선상 혹은 자상이며, 골은 다양한 방향이다. 칠엽수科의 화분을 발아구의 형태와 표면무늬에 의하여 *hippocastanum*형, *indica*형 그리고 *punduana*형의 3가지 화분형으로 나눌 수 있었다.

INTRODUCTION

Hippocastanaceae is a small family nearly allied to Aceraceae, but distinguished by irregular obliquely zygomorphic flowers and tricarpellary ovary. It contains two genera with 20 species and one variety distributed throughout the north-temperate zone, but mainly in U.S, on the North American continent(Rendle, 1952). *Aesculus hippocastanum*(Horse Chestnut, Buckeye) is the only extant European representative at the present day, growing wild in the mountains of northern Greece, Albania, Bulgaria and Yugoslavia(Greuter *et al.*, 1986). There have been various opinions in the past, whether the buckeyes represent a distinct family, or rather a subfamily within Sapindaceae. Early workers(Pax, 1895 ; Bessey, 1915 ; Rendle, 1952) recognized Hippocastanaceae as a distinct family from Sapindaceae, and most

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manuals currently used follow this interpretation.

The pollen morphology of *Aesculus* species has been studied by many authors : *A. hippocastanum* by Erdtman(1952), Maurizio and Louveaux(1960), Faegri and Iverson(1975), Nilsson *et al.*(1977), Horac and Jager(1979), Nakamura(1980), Kim (1981), Heath(1984) ; *A. indica* by Nair(1962) ; *A. glabra* by Shimakura (1973) ; *A. parviflora* by Tatzreiter(1985) ; and *Aesculus spp.* by Pozhidaev (1995). Hsiech(1979) reported that the morphology of *Aesculus* pollen shows some taxonomical differences. It was also noticed that morphological characteristics of the pollen can be used to distinguish taxonomical groups.

There is only limited information on pollen morphology of several species included in Hippocastanaceae, and some of these species have not been studied as yet. Therefore, a study of pollen morphology of 20 species and one variety of two genera of Hippocastanaceae was undertaken by light microscopy(LM), scanning electron microscopy(SEM) and transmission microscopy(TEM), to advance the understanding of the taxonomy of this family.

MATERIAL AND METHODS

Most materials were obtained from the herbarium of the Swedish Museum of Natural History, Stockholm(S). Attempts were made to use specimens which had been annotated by some specialists. Fresh material of three species was collected by the senior author in Korea. Both the fresh and herbarium material was acetolysed. In total, 20 species and one variety of two genera were investigated. All LM observations were made on Olympus BH-2 microscope equipped with an apochromatic oil-immersion objective(D plan×100= N.A.I. 25) and periplan eyepieces(WHK×10). Measurements were made using an oil-immersion objective. Twenty or more pollen grains per species were measured. Exine thickness was measured in the centre of mesocolpia, sculptural pollen. For scanning electron microscopy(SEM), acetolysed pollen grains were suspended in a drop of absolute alcohol and transferred to brass stubs. Gold coating was done for 7 minutes using a Fine Coat ION Sputter JFC-1100. Scanning micrographs were taken with a Jeol JSM 25 S 11 microscope using 1 for FP 4 film. For transmission electron microscopy(TEM), herbarium pollen were fixed in a phosphate buffered 3% glutaraldehyde solution(pH 7.4) for 2 days at room temperature. After postfixation in 2% OsO_4 solution for 12 hr and dehydration in an ethyl alcohol series and absolute acetone the samples were finally embedded in Epon. Ultrathin sections were cut with a glass knife on a Reicheert-Jung ultratome, poststained with 1% uranyl acetate and 1% lead citrate and examined under a Tesla BS-500 transmission microscope.

The terminology used follows Erdtman(1969), and Praglowski and Punt(1973).

RESULTS AND DISCUSSION

In Hippocastanaceae, pollen grains are monads, symmetrical, 3-colporate or 3-colpate. The polar axis length is 18~38㎛, equatorial width 11~28㎛, P/E 1.14~2.18. Shape in polar view rounded is to rounded triangular, in lateral view rounded to elliptic. Exine 0.8~2.2㎛ thick, distinctly striate or spinulose, lirae in various directions, sexine slightly thicker than nexine or of equal thickness. Apertures compound, colpi 18~31㎛ long, width very variable(1.8~11.5㎛), with tapered or blunt ends ; spaced pointed spinules ; ora 3.1~5.8×3.0~6.8㎛, circular or lolongate (Table 1, Plate Ⅰ, Ⅱ).

Three pollen types can be recognized which are described below.

1. *Hippocastanum* type(Plate Ⅰ. Fig. 1~2, Plate Ⅱ. Fig. 1~6)

This type is the most common and most variable in pollen characteristics. Pollen grains are subprolate to prolate ; colpi comparatively long and narrow, approximately 18~31㎛ long ; colpus mainly tapered, rarely blunt, usually equatorially constricted, colpus membrane beset with sparcely spaced, distinctly pointed spinules, occasionally irregular rupturing of the membrane covering the os membrane studded with a few process, os 3.1~5.8×3.0~6.8㎛ almost lolongate, spinules

Table 1. Pollen morphological data of Hippocastanaceae

Taxa	Pollen grains(㎛)		P / E	Equatorial view	Aperture(㎛)			Exine		Pollen type
	PL	EW			Colpus		Ora	Thickness (㎛)	Sculpture pattern	
					Width	Length	W L			
Aesculus arguta	31(28-35)	21(17-28)	1.48(1.21-1.79)	prolate	3.0(2.5-3.5)	26(24-29)	4.7x4.9	1.9(1.5-2.0)	striate	I
A. austrina	34(31-36)	24(20-27)	1.45(1.22-1.57)	prolate	3.0(2.5-3.0)	28(25-31)	4.3x4.3	2.0(1.9-2.1)	striate	I
A. californica	29(25-31)	18(16-21)	1.61(1.25-1.81)	prolate	3.5(2.0-4.0)	24(21-27)	3.7x3.7	1.8(1.7-1.9)	striate	I
A. carnea	31(27-34)	20(17-23)	1.55(1.34-1.79)	prolate	3.4(3.0-4.0)	26(22-29)	4.8x3.9	2.1(1.9-2.2)	striate	I
A. chinensis	31(28-38)	21(20-23)	1.46(1.22-1.81)	prolate	2.9(2.0-3.0)	27(24-34)	4.3x4.5	1.9(1.8-2.1)	striate	I
A. discolor	26(25-27)	20(19-22)	1.30(1.18-1.35)	subprolate	3.0(2.8-3.2)	22(21-24)	4.4x4.2	1.9(1.8-2.0)	striate	I
A. flava	33(31-34)	22(20-24)	1.49(1.38-1.57)	prolate	4.2(4.0-5.0)	28(26-29)	5.8x6.8	1.9(1.8-2.1)	striate	I
A. glabra	27(22-30)	19(12-22)	1.42(1.14-1.83)	prolate	3.0(2.4-4.0)	21(18-26)	3.8x4.7	1.9(1.7-2.0)	striate	I
A. hippocastanum	28(25-32)	18(15-21)	1.56(1.33-2.13)	prolate	4.2(3.0-5.0)	24(20-28)	4.6x5.3	1.7(1.4-1.9)	striate	I
A. humilis	28(27-29)	20(18-21)	1.40(1.29-1.61)	prolate	3.0(2.5-3.5)	23(22-24)	3.9x4.9	1.8(1.7-1.9)	striate	I
A. indica	33(30-35)	21(19-23)	1.57(1.35-1.84)	prolate	9.9(8.0-11.5)	27(25-29)	4.3x4.4	1.9(1.7-2.0)	striate	II
A. lutea	27(24-28)	18(16-19)	1.50(1.26-1.75)	prolate	3.0(2.5-3.5)	23(19-25)	3.9x3.9	1.8(1.7-1.9)	striate	I
A. macrostachya	25(24-26)	13(11-15)	1.90(1.67-2.18)	prolate	2.0(1.8-2.2)	21(20-23)	3.1x3.0	1.5(1.4-1.7)	striate	I
A. neglecta	28(26-29)	21(20-23)	1.33(1.18-1.45)	subprolate	3.3(3.5-4.5)	23(22-25)	4.2x4.4	1.8(1.6-2.0)	striate	I
A. octandra	29(27-31)	19(17-21)	1.53(1.38-1.76)	prolate	3.0(2.5-3.4)	24(22-25)	4.0x4.0	2.0(1.9-2.1)	striate	I
A. pavia	30(29-31)	20(19-22)	1.49(1.32-1.63)	prolate	2.6(2.0-3.0)	26(24-27)	4.5x4.5	1.9(1.8-2.0)	striate	I
A. paviflora var. *serotina*	21(18-24)	15(14-19)	1.40(1.27-1.68)	prolate	2.4(2.2-2.7)	22(20-25)	3.6x3.8	1.7(1.5-1.9)	striate	I
A. punduana	27(25-29)	18(16-20)	1.50(1.35-1.69)	prolate	6.4(6.0-7.2)	23(21-24)	3.9x3.8	1.8(1.7-2.0)	spinulose	III
A. rubicunda	26(23-29)	15(12-16)	1.73(1.60-2.17)	prolate	3.0(2.5-3.5)	22(18-25)	4.2x4.4	1.8(1.7-1.9)	striate	I
A. turbinata	26(25-27)	16(15-17)	1.63(1.53-1.80)	prolate	3.0(2.8-3.2)	23(21-24)	4.1x4.1	0.9(0.8-1.0)	striate	I
Billia columbiana	33(32-35)	23(22-25)	1.43(1.28-1.55)	prolate	3.4(3.0-4.0)	27(25-29)	5.2x5.2	1.8(1.7-1.9)	striate	I

Abbreviations : PL = Polar axis length EW = Equatorial width P / E = Polar axis length / Equatorial width
I = *Hippocastanum* type II = *Indica* type III = *Punduana* type W = Width L = Length

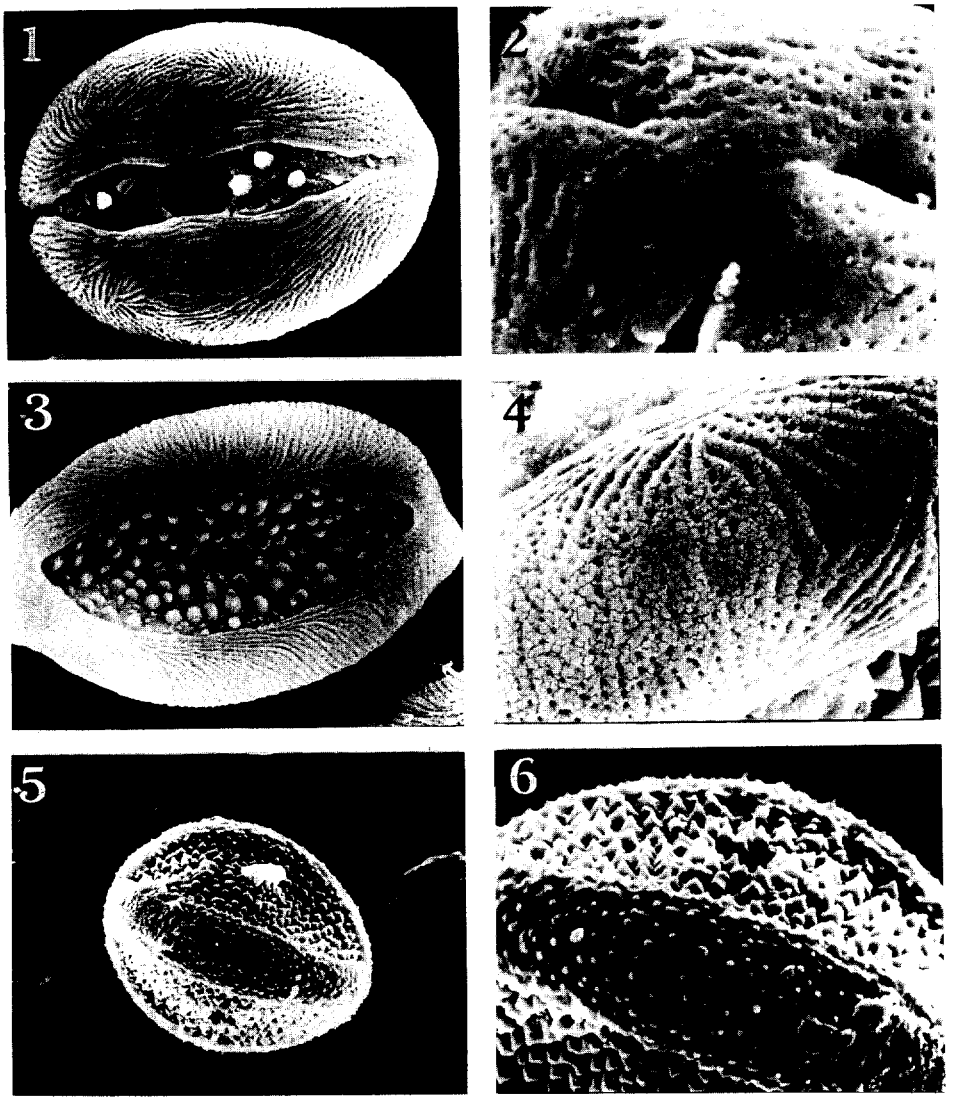

Plate I. Scanning electron microscopy(SEM) of pollen grains of the genus *Aesculus* L.

Figs. 1~2. *Aesculus hippocastanum* L.
1. Pollen grain in equatorial view, showing constricted colpus (×4,000)
2. Part of sculpture pattern, showing striate (×15,000)

Figs. 3~4. *Aesculus indica* Coelbr.
3. Pollen grain in equatorial view, showing broad colpus (×4,000)
4. Part of sculpture pattern, showing striate (×15,000)

Figs. 5~6. *Aesculus punduana* Wall.
5. Pollen grain in equatorial view, showing broad colpus (×3,500)
6. Part of sculpture pattern, showing spinulose (×11,000)

often more densely spaced over the os region, spinule size on the colpus membrane approximately 0.6~1.0㎛ wide, 0.6~1.3㎛ high. Exine striate ; Lirae usually transversely oriented in the central part of mesocolpia, paralleled at the colpus margin ; at the poles not always evident,

lirae width approximately 0.2~0.3μm ; striae with numerous perforations.

The following species are characterized as being of *Hippocastanum* type :

1. *Aesculus arguta* 2. *A. austrina*
3. *A. californica* 4. *A. carnea*
5. *A. chinensis* 6. *A. discolor*
7. *A. flava* 8. *A. glabra*
9. *A. hippocastanum* 10. *A. humilis*
11. *A. lutea* 12. *A. macrostachya*
13. *A. neglecta* 14. *A. octandra*
15. *A. pavia* 16. *A. paviflora* var. *serotina*
17. *A. rubicunda* 18. *A. turbinata*
19. *Billia columbiana*

2. *Indica* type(Plate Ⅰ. Fig. 3~4)

Pollen grains are prolate, polar axis length 30~35(average 33)μm, equatorial width 19~23(21)μm, P/E 1.35~1.84(1.57). Exine 1.7~2.0(1.9)μm thick. Colpi approximately 9.9μm wide, much wider than in the other species of Hippocastanaceae ; colpi not constricted in the equatorial region, ends comparatively blunt and rounded ; colpus membrane studded with evenly spaced spinules ; spinule size on the colpus membrane approximately 0.5~1.0μm wide, 0.4~1.1μm high. Lirae width 0.2~0.3μm ; colpus width much wider than ora width, os lolongate, situated inside colpus outline.

Species included : *Aesculus indica*

3. *Punduana* type(Plate Ⅰ. Fig. 5~6)

Pollen grains prolate, polar axis length 25~29(average 27)μm, equatorial width 16~20(18)μm, P/E 1.35~1.69(1.50). Exine 1.7~2.0(1.8)μm thick. Colpi approximately 6.4μm wide, much wider than in the other species except *Aesculus indica* of Hippocastanaceae ; colpi not constricted in the equatorial region, ends comparatively blunt or rounded ; colpus membrane studded with evenly spaced spinules, spinule size on membrane 0.1~0.3μm wide, 0.4~0.8μm high, spinule size on the surface 0.1~0.2μm wide and high.

Species included : *Aesculus punduana*

Key to the pollen types

1. Colpus constricted in the equatorial region ..... *Hippocastanum* type(Pollen type Ⅰ)
1. Colpus not constricted in the equatorial region
2. Exine striate ..... *Indica* type(Pollen type Ⅱ)
2. Exine spinulose ..... *Punduana* type(Pollen type Ⅲ)

Data in Table 1 indicate a degree of variability in characteristics listed among the different taxa, with a wide overlapping in their range of variation. *Aesculus austrina* has the highest value for polar axis length and equatorial width(P=34μm, E=24μm), and *A. paviflora* var. *serotina* the lowest(P=21μm, E=15μm). The species with the longest pollen grain is also the species with the widest pollen grain. However it is impossible to identify the species or pollen types on the basis of pollen size only(polar axis length or equatorial width) since they overlap.

The width of colpus aperture is 2.0~4.2μm except for the *A. indica*(9.9μm) and *A. punduana* (6.4μm). It is highly variable depending on the species and colpus width. For *A. indica*, it is approximately 5 times the width of *A. marcrostachya*. However, colpus length is less variable (21~28μm) compared to colpus width. Colpus width is an important characteristic to provide for the pollen key on the level of types in this family. Most of sculpture pattern is striate except spinulose on *A. punduana*. Also, sculpture pattern can be an evidence to provide for the pollen key, too.

Morphological differences among sections are winter-buds, leaf sessile and flower colours etc. In case of pollen morphology of *Aesculus hippocastanum*, *A. carnea* and *A. turbinata* of *Hippocastanum* type coincide with winter bud of section *Hippocastanum*. However, it was very difficult to classify on the pollen morphology of some sections since there are high similarities in pollen morphology among species of some sections and *Hippocastanum* type.

Pollen morphology of the monotypic genus *Billia*, the second Hippocastanaceae genus, is

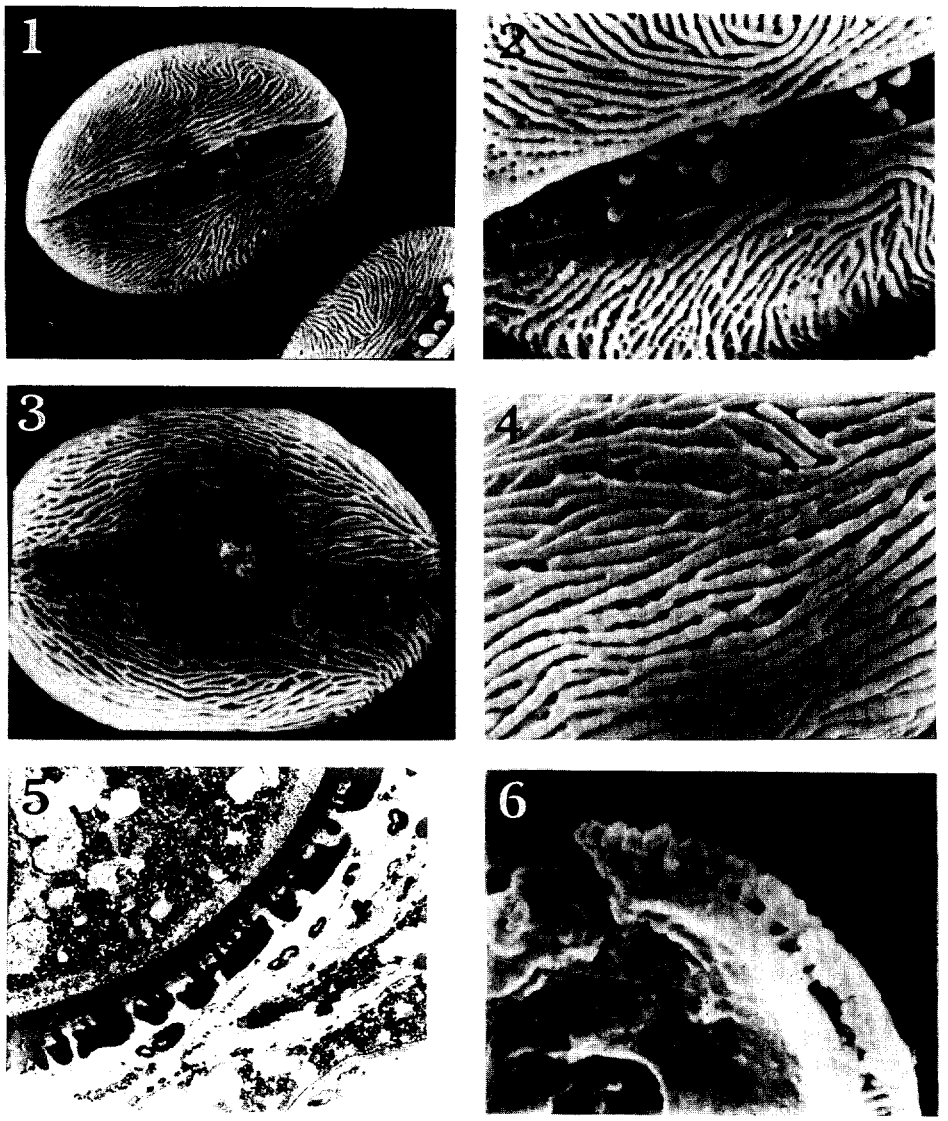

Plate II. Scanning electron microscopy(SEM) and transmission microscopy(TEM) of pollen grain of Hippocastanaceae

Fig. 1~2. *Aesculus pavia*
1. Pollen grain in equatorial view, showing colpus with pore (SEM × 3,500)
2. Part of colpus, showing lolongate (SEM × 11,000)

Fig. 3~5. *Billia columbiana*
3. Pollen grain in equatorial view, showing colpus with pore (SEM × 4,000)
4. Part of sculpture pattern with perforations (SEM × 11,000)
5. Structure of wall, showing wall stratification (TEM × 7,000)

Fig. 6. Ultrathin section of *Aesculus californica* (SEM × 11,000)

very similar to the pollen of the species of *Hippocastanum* type(Plate II. Fig. 3~5).

The structure of the pollen wall of Hippocastanaceae presented typical angiosperm pollen wall

stratification consisting of a columellate ectexine, a two-three-layered, fibrillar intine and an endexine considerably thickened around the endoaperture(Plate Ⅱ. Fig. 5~6).

Hardin(1957a) suggested that Hippocastanaceae derived independently and on different lines from Sapindaceae. The pollen morphology of this family appears to support this suggestion since there are differences in pollen morphology(colpus type and sculpture pattern) between Sapindaceae, Anacardiaceae and Aceraceae(Erdtman, 1952 ; Kim, 1981). Also, Hardin(1957b) suggested that *Aesculus* has derived from an ancestral form similar to *Billia* and apparently represents the extreme in that evolutionary direction. From pollen morphological view, *Billia columbia*(the only species of the genus) is similar to *Aesculus* with the exception of *A. indica* and *A. punduana*, and also this study provides support for Hardin's (1957a) report.

The classification of Hippocastanaceae is complicated. Hardin(1957a, 1960) included 13 species arranged in five sections(*Aesculus*, *Calothyrsus*, *Parryanae*, *Macrothyrsus* and *Pavia*). Rehder (1940) distinguished four sections(*Hippocastanum*, *Calothyrsus*, *Macrothyrsus*, *Pavia*) and two series (*Octandrae* and *Eupaviae*) within the section *Pavia*. Krussman(1960) distinguished the same four sections(*Hippocastanum*, *Calothyrsus*, *Macrothyrsus*, *Pavia*) and two groups - *Octandrae* and *Eupaviae*. Section *Pavia* has been separated from *Hippocastanum*. However, pollen morphology of *A. pavia* appears very similar to *Hippocastanum* type(Plate Ⅱ. Fig. 1~2) and can not be separated into an independent section on palynological view.

From palynological point of view *A. indica* and *A. punduana* appear to be the most distinctive from all species of Hippocastanaceae(Table 1). Genus *Billia* is very similar to *Aesculus*. Taxonomic relations in this family need to be reconsidered, and other factors should be used for further investigation. In particular, the use of genetic markers(for example isozyme or PCR-based DNA markers) will be invaluable in addressing the taxonomic relationships of the Hippocastanaceae. Genetic markers present qualitative variation, mainly neutral in the evolutionary sense, and can provide a different set of characters for use in systematics. Genetic markers coupled with the quantitative data revealed by the pollen morphology traits will form a basis for a unified approach in the study of the complex taxonomy of Hippocastanaceae.

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Specimen investigated

Aesculus L.

A. arguta Buckl., U.S.A. Kansas, McGregor E.372(S).

A. austrina Amall, U.S.A. Texas, Warnock 46019(S).

A. californica Spach Nutt., Korea, Chungnam, Kim s.n.

A. carnea Hayne, Germany, Schlesien, Griesche s.n.

A. chinensis Bge., China, Tchenkeou-Tin, Farges 42(S).

A. discolor Pursh., U.S.A., Texas, Shinners 22390(S).

A. flava Ait., U.S.A., N. Carolina, Lidman 1289(S).

A. glabra Willd., U.S.A., Rich Mountain, Demaree 55183(S).

A. hippocastanum L., Korea, Chungnam, Kim s.n.

A. humiliss Lodd., U.S.A., Georgia, Smith s.n.(S).

A. indica Coelbr., India, Siwalik and Janusar Divisions, Sharma 29(S).

A. lutea Wangenheim., German, Berlin, Lindman s.n.(S).

A. macrostachya Michx., U.S.A., North Amer., Novlfayy s.n.(S).

A. neglecta Lindl., Sweden, Visby, Johansson s.n.(S).

A. octandra Marsh., U.S.A. Penn., Brumbach 7180(S).

A. pavia L., U.S.A., Seorgia, Laurence 637 (S).

A. paviflora var. Serotina Rehd., U.S.A., Alabama, Wolf 2150

A. punduana Wall., India, Jalpaiguri, Parker 3204(S).

A. rubicunda Dc., Sweden, Malmo, Sundstedt s.n.(S).

A. turbinata Bl., Korea, Kyunggi, Kim s.n.

Billia Pl. & Lindl.

B. columbiana Pl. & Lindl., Columbia, Dephdel, Cuahecasas 22185(S).