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RESEARCH ARTICLE

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Taxonomic reconsideration of *Disporum luzoniense* (*Liliaceae* s.l.) using flavonoid characters

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Key words

Disporopsis
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Philippines
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taxonomy

Abstract Phytochemical characters of the plants that have been treated as *Disporum luzoniense* (Merrill & Merritt 1910) or *Disporopsis fuscopicta* (Jessop 1979) in the Philippines were compared with those of *Disporum kawakamii* and *Disporopsis fuscopicta* in Taiwan. The present phytochemical study revealed that *Disporum kawakamii* had luteolin, apigenin and chrysoeriol as free state, while these flavonoids were not detected in the Philippine plant and *Disporopsis fuscopicta* from Taiwan. Moreover, flavone *O*-glycosides were isolated from *Disporum kawakamii*, while flavone *C*-glycoside was isolated from the Philippine plant and *Disporopsis fuscopicta* from Taiwan. In conclusion, the present study suggests that the Philippine plant is chemotaxonomically related to *Disporopsis* and this agrees with the taxonomic treatment of Jessop (1979).

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INTRODUCTION

The genus *Disporum* Salisb. (*Liliaceae* s.l.; *Colchicaceae* sensu, APG II 2003) consist of 22 species primarily distributed in the temperate zone of eastern Asia (Kawano & Takasu 2004). On the other hand, the genus *Disporopsis* Hance (*Liliaceae* s.l.; *Convallariaceae*, APG II 2003) consists of 6 species that are primarily distributed in eastern Asia (Liang & Tamura 2000). In 1910 Merrill (Merrill & Merritt 1910) described *Disporum luzoniense* based on a type specimen collected from Benguet Province, Luzon, the Philippines (*E.D. Merrill 6619*, deposited in US). Thereafter, Jessop (1979) identified the Benguet plant as *Disporopsis fuscopicta* Hance based on morphological characters. His taxonomic treatment was taken up by Kumar & Brandham (1980) and Hara (1988).

Flavonoid compounds based on a fifteen-carbon skeleton consist of two phenyl rings (A- and B-rings) connected by a three-carbon bridge (C-ring). In most cases, the flavonoids are present as glycosides in flowers, leaves, stems or roots. They were divided into several classes, i.e., flavones, flavonols, flavanones, anthocyanins, etc. Numerous kinds of flavonoids have been found in plants by the combination of additional hydroxyl, methoxyl, methyl and/or glycosyl groups. At the present, more than 7 000 kinds of flavonoids have been reported as natural products (Andersen & Markham 2006). These flavonoids have frequently been used as chemotaxonomic markers (e.g. Iwashina et al. 1995, Yamazaki et al. 2007).

The aim of the present study is to assess the taxonomic status of the plant previously labelled as *Disporum luzoniense* from the Philippines by comparing the phytochemical characters with morphological and cytological observation and to compare this plant with specimens labelled as *Disporum* and *Disporopsis* from Taiwan.

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MATERIAL AND METHODS

Plant materials

Plant materials from the experimental greenhouse of Tsukuba Botanical Garden, National Museum of Nature and Science were used for morphological, phytochemical and cytological study. Voucher specimens are deposited in the herbarium of the National Museum of Nature and Science (TNS).

Morphological characters

The following morphological characters of the specimens were observed in this study: presence/absence of corona inside of perianth and presence/absence of rhizomes.

One of two plants collected from Taiwan was morphologically identified as *Disporum kawakamii* Hayata in having an umbel type of inflorescence with few flowers (Fig. 1a), no corona inside the perianth and very thin or no rhizomes at all (Fig. 1d, Ying 2000); and another was identified as *Disporopsis fuscopicta* Hance var. *arisanensis* (Hayata) S.S.Ying in having an axillary inflorescence (Fig. 1c), well-developed rhizomes (Fig. 1f), and presence of a corona inside the perianth (Fig. 2, Ying 2000).

The floral and rhizome morphologies of the plant from the Philippines were similar to those of *Disporopsis fuscopicta* var. *arisanensis*, and were also consistent with the description of *Disporum luzoniense*.

Chromosome study

Root tips were harvested, pretreated in 2 mM 8-hydroxyquinoline at 20 °C for 2h, and fixed in acetic ethanol (1 : 3) at 4 °C for 2h. The fixed root tips were macerated in 2 : 1 mixture of 1N hydrochloric acid and 45 % acetic acid at 60 °C for 10 seconds. Somatic chromosomes at mitotic metaphase were stained in 2 % aceto-orcein at 20 °C for 2h, and spread by the standard squash method.

Flavonoid extraction and isolation

Fresh leaves of *Disporum kawakamii* (25 g), *Disporum luzoniense* from the Philippines (129 g) and *Disporopsis fuscopicta* var. *arisanensis* (45 g) were extracted with methanol. The flavonoids were isolated by preparative paper chromatography

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Fig. 1 Plant materials. a, d. *Disporum kawakamii*; b, e. Philippine plant; c, f. *Disporopsis fuscopicta* var. *arisanensis*. — Scale bar = 3 cm.

Fig. 2 Corona inside of perianth of Philippine plant. — Scale bar = 1 cm.

using solvent systems: BAW (*n*-BuOH/HOAc/H_2O = 4 : 1 : 5, upper phase), 15 % HOAc (glycosides) or 50 % HOAc (aglycones) and then BEW (*n*-BuOH/EtOH/H_2O = 4 : 1 : 2.2). Their isolated flavonoids were purified by Sephadex LH-20 column chromatography using solvent system: 70 % MeOH.

HPLC and PC survey of crude extracts

The MeOH extracts were surveyed for flavonoid composition by Shimadzu HPLC systems (Shimadzu Co., Japan) using Pegasil ODS (I.D. = 6.0 × 150 mm (Senshu Scientific Co., Japan)) at a flow rate of 1.0 mL min^{-1}, injection: 10 µl, detection wavelength of 190–700 nm, and eluents of MeCN/H_2O/H_3PO_4 (35 : 65 : 0.2) (Solvent I) for crude extracts and aglycones and MeCN/H_2O/H_3PO_4 (22 : 78 : 0.2) (Solvent II) for glycosides. Two-dimensional paper chromatography (2D-PC) was performed using solvent systems: BAW (1st) and 15 % HOAc (2nd).

Identification of flavonoids

The isolated compounds were identified by UV spectral survey according to Mabry et al. (1970), LC-MS, acid hydrolysis (in 12 % aq.HCl, 100 °C, 30 min), and direct TLC and HPLC comparisons with authentic specimens. TLC, UV, LC-MS and HPLC data of the isolated compounds were as follows.

Luteolin (**1**). TLC: R_f 0.88 (BAW), 0.03 (15 % HOAc), 0.91 (BEW); UV - dark purple and UV/NH_3 - bright yellow. HPLC: retention time (Rt) 5.73 min (Solvent I). UV: λ_{max} (nm) MeOH 255, 266sh, 349; +NaOMe 268, 326, 403 (inc.); +$AlCl_3$ 272, 422; +$AlCl_3$/HCl 257, 273sh, 296, 359, 385sh; +NaOAc 267, 396; +NaOAc/H_3BO_3 262, 373. LC-MS: *m/z* 285 $[M-H]^-$.

Luteolin 7-*O*-glucoside (**4**). TLC: R_f 0.34 (BAW), 0.07 (15 % HOAc), 0.54 (BEW); UV - dark purple and UV/NH_3 - bright yellow. HPLC: Rt 6.01 min (Solvent II). UV: λ_{max} (nm) MeOH 256, 268sh, 351; +NaOMe 268, 394 (inc.); +$AlCl_3$ 272, 417; +$AlCl_3$/HCl 262, 274sh, 293, 357, 383sh; +NaOAc 260, 405; +NaOAc/H_3BO_3 258, 372. LC-MS: *m/z* 447 $[M-H]^-$, 285 [M-glucosyl-H]$^-$.

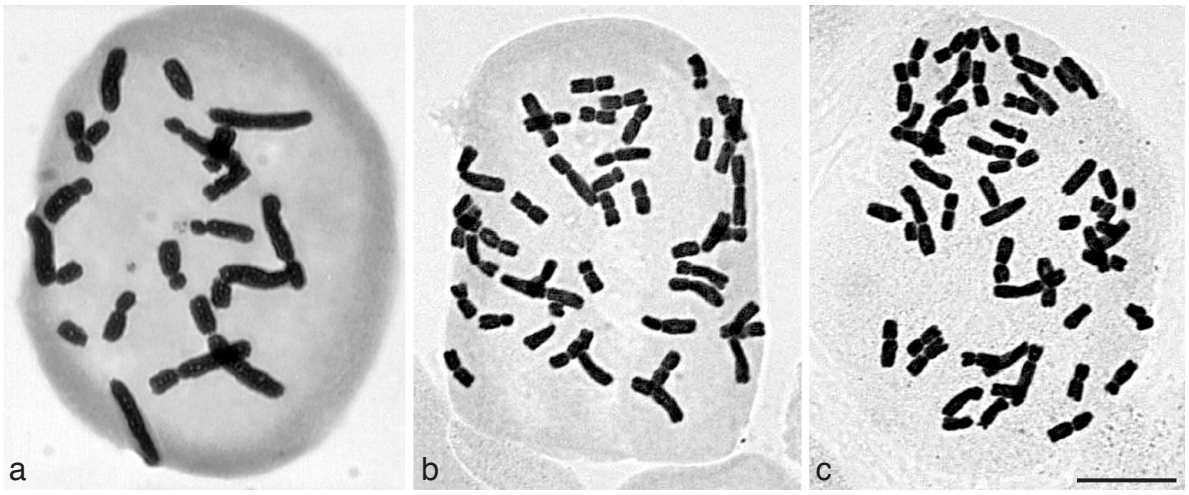

Fig. 3 Chromosomes stained with aceto-orcein at mitotic metaphase of plants investigated. a. *Disporum kawakamii*; b. Philippine plant; c. *Disporopsis fuscopicta* var. *arisanensis*. — Scale bar = 10 µm.

Luteolin 4´-*O*-glucoside (**5**). TLC: R_f 0.61 (BAW), 0.08 (15 % HOAc), 0.66 (BEW); UV and UV/NH_3 - dark purple. HPLC: Rt 8.90 min (Solvent II). UV: λ_{max} (nm) MeOH 269, 335; +NaOMe 267, 375 (dec.); +$AlCl_3$ 277, 293sh, 354, 380sh; +$AlCl_3$/HCl 277, 293sh, 351, 375sh; +NaOAc 269, 374; +NaOAc/H_3BO_3 270, 341. LC-MS: *m/z* 447 $[M-H]^-$, 285 [M-glucosyl-H]$^-$.

Luteolin 7-*O*-diglucoside (**6**). TLC: R_f 0.61 (BAW), 0.08 (15 % HOAc), 0.66 (BEW); UV - dark purple and UV/NH_3 - bright yellow. HPLC: Rt 4.49 min (Solvent II). UV: λ_{max} (nm) MeOH 255, 268, 348; +NaOMe 268, 392 (inc.); +$AlCl_3$ 273, 425; +$AlCl_3$/HCl 265, 275sh, 295, 360, 385; +NaOAc 261, 405; +NaOAc/H_3BO_3 259, 373. LC-MS: *m/z* 609 $[M-H]^-$.

Luteolin 7-*O*-rhamnosylglucoside (**7**). TLC: R_f 0.36 (BAW), 0.11 (15 % HOAc), 0.46 (BEW); UV - dark purple and UV/NH_3 - bright yellow. HPLC: Rt 5.50 min (Solvent II). UV: λ_{max} (nm) MeOH 255, 265sh, 349; +NaOMe 268, 390 (inc.); +$AlCl_3$ 272, 425; +$AlCl_3$/HCl 262, 274, 296, 361, 385sh; +NaOAc 260, 405; +NaOAc/H_3BO_3 259, 374. LC-MS: *m/z* 593 $[M-H]^-$.

Apigenin 6-*C*-hexoside-8-*C*-pentoside or 6-*C*-pentoside-8-*C*-hexoside (**8**). TLC: R_f 0.21 (BAW), 0.41 (15 % HOAc), 0.24 (BEW); UV - dark purple and UV/NH_3 - orange. HPLC: Rt 4.50 min (Solvent II). UV: λ_{max} (nm) MeOH 273, 333; +NaOMe 283, 334, 402 (inc.); +$AlCl_3$ 281, 306, 351, 380; +$AlCl_3$/HCl 280, 304, 347, 380; +NaOAc 283, 395; +NaOAc/H_3BO_3 285, 319, 354, 415. LC-MS: *m/z* 563 $[M-H]^-$.

RESULTS AND DISCUSSION

Chromosome numbers

The plant of *Disporopsis fuscopicta* var. *arisanensis* and the plant labelled as *Disporum luzoniense* had the same chromosome number of 2n = 40, which agrees with the findings of Chang & Hsu (1974) and Kumar & Brandham (1980) (Table 1, Fig. 3b, c). The chromosome number corresponds with the basic chromosome number of x = 20 in the genus *Disporopsis* (Darlington & Wylie 1955, Chang & Hsu 1974, Hong & Zhu 1990). On the other hand, *Disporum kawakamii* had a somatic chromosome number of 2n = 16 (Table1, Fig. 3a). This number agrees with the findings of Chao et al. (1963), Chang & Hsu (1974) and Tamura et al. (1992).

Previously Kumar & Brandham (1980) detected the chromosome number of 2n = 40 for a plant collected from the type locality of *Disporum luzoniense*, and then cytotaxonomically supported Jessop (1979). Also the present cytological investigation upholds Jessop's taxonomic treatment (1979) and also agrees with the findings of Kumar & Brandham (1980).

Phytochemical analysis and conclusion

Five major flavonoids were isolated from *Disporum kawakamii* (Table 2). They were completely identified as luteolin (**1**), luteolin 7-*O*-glucoside (**4**), luteolin 4´-*O*-glucoside (**5**), luteolin 7-*O*-diglucoside (**6**) and luteolin 7-*O*-rhamnosylglucoside (**7**).

Table 1 Comparison of morphological and cytological characters of plant materials studied.

Species	Inflorescence	Rhizome	Corona	Chrom. no. (2n)
Disporum kawakamii (Hsinchu, Taiwan; GK 5207)	terminal	absent	absent	16
Disporum luzoniense sensu Merrill/ *Disporopsis fuscopicta* sensu Jessop (Luzon, Philippines; YS 346)	axillary	present	present	40
Disporopsis fuscopicta var. *arisanensis* (Ilan, Taiwan, YS 392)	axillary	present	present	40

Table 2 Flavonoid characters.

Species	Aglycones			Glycosides				
				O-glycosides				*C*-glycoside
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Disporum kawakamii	+	+	+	+	+	+	+	–
Disporum luzoniense/*Disporopsis fuscopicta* (Philippines)	–	–	–	–	–	–	–	+
Disporopsis fuscopicta var. *arisanensis*	–	–	–	–	–	–	–	+

1 = luteolin; **2** = apigenin; **3** = chrysoeriol; **4** = luteolin 7-*O*-glucoside; **5** = luteolin 4´-*O*-glucoside; **6** = luteolin 7-*O*-diglucoside; **7** = luteolin 7-*O*-rhamnosylglucoside; **8** = apigenin 6-*C*-hexoside-8-*C*-pentoside or 6-*C*-pentoside-8-*C*-hexoside.

Furthermore two minor flavonoids were inferred as apigenin (**2**) and chrysoeriol (**3**) by HPLC comparisons with authentic samples, but the two minor flavonoids could not be completely identified for lack of enough samples.

Previously a chemotaxonomic study has been done on three Asian *Disporum* species, e.g., *D. calcaratum*, *D. cantoniense* and *D. sessile*, and the three species revealed that their main flavonoids were luteolin glycosides (Williams et al. 1993). In the present survey, main flavonoids were also four luteolin glycosides (**4**, **5**, **6** and **7**) in *Disporum kawakamii* following Williams et al. (1993). However, out of five major flavonoids detected (**1**, **4**, **5**, **6** and **7**), luteolin 7-*O*-rhamnosylglucoside (**7**) in *D. kawakamii* was different from that of luteolin 7-*O*-rutinoside (i.e. 7-*O*-rhamnosyl-(1→6)-glucoside) in the three *Disporum* species (Williams et al. 1993). Further phytochemical study is necessary for clarifying chemotaxonomic relationships in the genus *Disporum*.

On the other hand, only one flavone *C*-glycoside (**8**) was isolated from the Philippine plant and *Disporopsis fuscopicta* var. *arisanensis* in Taiwan (Table 2), but any flavone *O*-glycosides and flavone aglycones were not isolated from the two plants differing from that of *Disporum kawakamii*.

The present phytochemical analysis reveals that the flavonoid composition of the plant labelled as *Disporum luzoniense* is completely consistent with *Disporopsis fuscopicta* var. *arisanensis* but distinctly different from those of *Disporum kawakamii* and the three *Disporum* species reported by Williams et al. (1993). In conclusion, the phytochemical study suggests that the Philippine plant that was previously identified as *Disporum luzoniense* is actually related to *Disporopsis.* Our phytochemical study result agrees with the taxonomic study of Jessop (1979) and Kumar & Brandham (1980).

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