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PHYLOGENETIC STUDIES OF TAIWAN FRESHWATER MUSSELS (BIVALVIA: UNIONIDAE)[1]

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Wen-Lung Wu (1982) Phylogenetic studies of Taiwan freshwater mussels (Bivalvia: Unionidae). *Bull. Inst. Zool., Academia Sinica* **21**(2): 145-153. The morphological and biochemical characters of three Taiwan freshwater mussels, *Unio douglasiae*, *Anodonta woodiana* and *Cristaria discoidea* are compared. In *A. woodiana* and *C. discoidea*, scars of anterior and posterior retractor-pedis muscles are incorporated in the scars of anterior and posterior adductor respectively, while those of *U. douglasiae* are separate. The shape of *U. douglasiae* is elongate, while *A. woodiana* and *C. discoidea* are oval. The hinge teeth of *U. douglasiae* are very well developed, but they are very weakly developed in *C. discoidea* and lacking in *A. woodiana*. However, the genetic distances estimated from the proteins show a somewhat difference in electrophoretic pattern. On the basis of muscle-scars, some conchometric characters and the band patterns of the soluble muscle proteins, the Taiwan freshwater mussels can be sorted into two groups: *U. douglasiae* in one and *A. woodiana* and *C. discoidea* in the other.

The molluscs of the family of Unionidae are widely distributed in freshwater meres and rivers of the world. Three species, *Unio douglasiae* (UD), *Anodonta woodiana* (AW) and *Cristaria discoidea* (CD), commonly occur in Taiwan. *U. douglasiae* has been reported from eastern Siberia, Korea, Japan, mainland China and Taiwan; *A. woodiana* from eastern Siberia, USSR, Korea, Japan and east Asia; *C. discoidea* from mainland China, Taiwan, and south-east Asia[16]. They are important edible bivalves in Taiwan[22], and frequently taken by house and pump from *Corbicular* culture ponds[21].

Previous accounts of Taiwan freshwater mussels have been published by Cuming[3], Adams[1], Pilsbry and Hirase[17], Matuda[12], Hayasaka and Tan[6], Horikawa[7,8], Kuroda[10], van der Schalic and Pace[19], Pace[16], and Wu[20,23]. The synoptic key for identifying the Taiwan unionid species was prepared by Pace[16]. However, some conchomorphometric characters used to distinguish species overlap to some extent. A more extensive comparative study on their muscle-scars, hinge teeth, shell form and electrophoretic characters is therefore needed for accurate species identification and elucidation of species interrelationship.

MATERIALS AND METHODS

Twenty specimens of three species each were used in this study. With the aid of vernier calipers, measurements of shell length (SL), shell height (SH) and shell breadth (SB) were made in millimetres. The hinge teeth region and the scars of the retractor-pedis,

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adductor, protractor-pedis were also examined.

Animals were dissected and the fresh adductor muscles were removed immediately. The muscle was homogenized with equal volume of phosphate buffer (KH_2PO_4-K_2HPO_4) (*I* 0.05, pH 7.5). The homogenate was centrifuged at 3,000 rpm for 15 minutes at 4°C. Extracts from the upper layer of liquid in the tube were prepared according to the formula of Nagai[13]. Bisacryalmide at 5% of the total concentration of acrylamide (*T* 5%) was the most satisfactory concentration. Tris-glycine buffer[15] was used as the tray buffer system. After 3-4 hours of running (SG-8, Slab Gel Electrophoresis, M & S Instruments Inc., Japan) at a current of 35 mA per two plates, the gels were stained with 1% Amido-Black 10B in 7% acetic acid and destained in 7% acetic acid. Genetic distance (*D*) was obtained from $D=-\ln I$, where *I* is the genetic identity derived from the formula of Nei[14].

RESULTS

Conchomorphometric proportion

The measurements of conchomorphometric proportions are given in Table 1. The ratio of shell height to shell length or the elongation index of *U. douglasiae* (1.97±0.20) is larger than that of *A. woodiana* and *C. discoidea*, but the ratios of shell breadth to shell length of the three species apparently overlap. The regression coefficients of shell height (Table 2) on shell length for the three species are close to one another. The linear regression equations indicate that the elongation index of *U. douglasiae* is the largest of the Taiwan freshwater mussels.

Muscle-scars, hinge teeth

The muscle-scar patterns of the left valve of the three species are shown in Fig. 1. The scar of retractor-pedis is clearly detached from the scar of the adductor in *U. douglasiae*, while it is incorporated in the scar of the adductor in *A. woodiana* and *C. discoidea*.

The hinge teeth of three species are in Plate 2. The hinge has well developed teeth in *U. douglasiae*, but they are weakly developed in *C. discoidea* and lacking in *A. woodiana*.

Electrophoresis of adductor muscle

On the basis of Taiwan specimens, the electrophoretic patterns of the three species are

TABLE 1

Ratios of shell height and breadth to length of Taiwan freshwater mussels. Values given are range, and mean±2SD in parentheses

Species / Shell measurement	*Anodonta woodiana*	*Cristaria discoidea*	*Unio douglasiae*
Height	1.36-1.84(1.63±0.24)	1.35-1.74(1.56±0.21)	1.78-2.15(1.97±0.20)
Breadth	2.05-3.29(2.69±0.68)	2.47-4.50(2.96±0.95)	2.60-3.13(2.81±0.30)

TABLE 2

The linear regression from shell height (SH, Y) and shell breadth (SB, Y) against shell length (SL, X) of *A. woodiana* (AW), *C. discoidea* (CD) and *U. douglasiae* (UD). The modal of linear regression is $Y=a+bx$, where *r* is correlation coefficience

	SL vs. SH			SL vs. SB		
	AW	CD	UD	AW	CD	UD
b	0.4859	0.4235	0.4547	0.2183	0.4883	0.3608
a	9.6839	12.4453	2.4663	11.6156	−8.1317	−0.1687
r	0.9815	0.8626	0.9857	0.9603	0.8613	0.9783

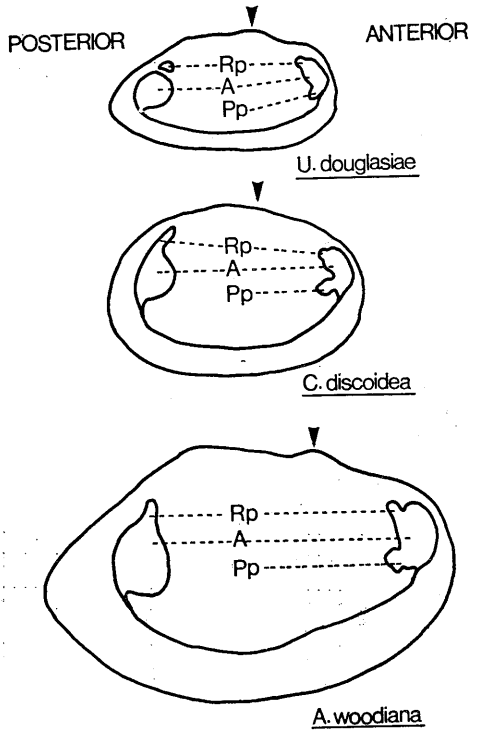

Fig. 1. The muscle scars of the Taiwan freshwater mussels. The position of umbo is indicated by an arrow. R_p, retractor-pedis; A, adductor; P_p, protractor-pedis.

very similar except for minor differences in bands 5, 6 and in the mobility of band 7 (Fig. 2). However, the estimated genetic distances differ among the three species; 0.0501, 0.4218 and 0.2818 for *A. woodiana vs. C. discoidea*, *A. woodiana vs. U. douglasiae*, and *C. discoidea vs. U. douglasiae*, respectively (Fig. 3); based on the data in Tables 3-4.

Species accounts

Key of Taiwan unionid species

1. Shell very elongate (elongation index about 2.0); scar of retractor-pedis detached from scar of adductor; hinge with well developed teeth in both valves ..... *Unio douglasiae*
   Shell oval (elongation index about 1.5); scar of retractor-pedis incorporated in scar of adductor; hinge lacking or with weakly developed teeth 2
2. Shell with postero-dorsal wings; umbones swollen; hinge lacking teeth ..... *Anodonta woodiana*
   Shell without postero-dorsal wings; umbones flat and usually worn away; hinge with weakly developed teeth ..... *Cristaria discoidea*

***Unio douglasiae* (Griffith and Pidgeon, 1834)**
(Plate 1)

Diagnosis: Shell height 1.78–2.15, shell breadth 2.60–3.13 in shell length (Table 1); umbo about 1/3 distance from the anterior end (Fig. 1), umbonal sculpture weak nodular radiating in zigzag lines. Hinge region with well developed teeth in both valves, usually two cardinal teeth in left valve and one in right valves (Plate 2). Scars of the anterior and posterior retractor-pedis detached from scars of anterior and posterior adductor respectively (Fig. 1).

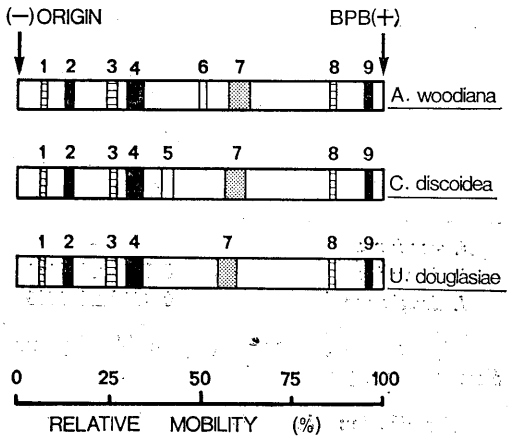

Fig. 2. Electrophoretic patterns of soluble muscle proteins of the Taiwan freshwater mussels.

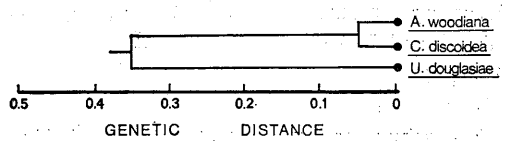

Fig. 3. Phylogenetic tree for the Taiwan freshwater mussels.

TABLE 3

Frequencies of protein bands on gels of Taiwan freshwater mussels

Protein variants	*A. woodiana* N*=20		*C. discoidea* N=20		*U. douglasiae* N=20	
1	20	1.0000	20	1.0000	18	0.9000
null	0	0	0	0	2	0.1000
2	20	1.0000	20	1.0000	20	1.0000
null	0	0	0	0	0	0
3	15	0.7500	8	0.4000	12	0.6000
3′	3	0.1500	7	0.3500	6	0.3000
null	2	0.1000	5	0.2500	2	0.1000
4	18	0.9000	17	0.8500	15	0.7500
null	2	0.1000	3	0.1500	5	0.2500
5	8	0.4000	16	0.8000	2	0.1000
null	12	0.6000	4	0.2000	18	0.9000
6	18	0.9000	6	0.3000	0	0
null	2	0.1000	14	0.7000	20	1.0000
7	17	0.8500	15	0.7500	2	0.1000
7′	2	0.1000	2	0.1000	16	0.8000
null	1	0.0500	3	0.1500	2	0.1000
8	20	1.0000	20	1.0000	20	1.0000
null	0	0	0	0	0	0
9	20	1.0000	20	1.0000	20	1.0000
null	0	0	0	0	0	0

* N, number of individuals examined.

TABLE 4

The genetic identity and genetic distance based on the frequencies of soluble proteins among Taiwan freshwater mussels

	A. woodiana	*C. discoidea*	*U. douglasiae*
A. woodiana	—	0.9535±0.0697	0.7977±0.1184
C. discoidea	0.0501±0.0252	—	0.8278±0.0987
U. douglasiae	0.4218±0.2703	0.2818±0.1713	—

Upper diagnol shows genetic identity among the three species and lower diagnol shows genetic distance among them.

Distribution in Taiwan: Found in both sandy and muddy substrata of freshwater ponds, widely distributed in Taiwan. The locations studied in Taiwan are listed below: Taipei City; Shinwu, Taoyuan Hsien; Lotung and Lanyang stream, Ilan Hsien; Hwalien Hsien; Shiesi and Lukang, Changhwa Hsien; Sunmoon lake, Nantou Hsien; Mailiao, Yunlin Hsien; Talin, Chiayi Hsien; Tainan City; Liuying and Chiangchun stream, Tainan Hsien; Chengkung, Taitung Hsien; Kaohsiung City; Meinung, Kaohsiung Hsien; Likang, Wulung stream, Tongkang, Hengchun and Lungluantan, Pintung Hsien (Fig. 4).

Remarks: Pilsbry and Hirase[17] named *Nodularia douglasiae taiwanica* as a new species in Taiwan. Many previous workers cited the same name until 1931. *Unio douglasiae taiwanica* was reported in Kuroda's paper[9]. Habe[4] considered that *U. douglasiae taiwanica* is a synonym of *U. douglasiae*. Hass[5] gave the following measurement range for *U. douglasiae*: shell length 64–80 mm, shell height 36–38 mm and shell

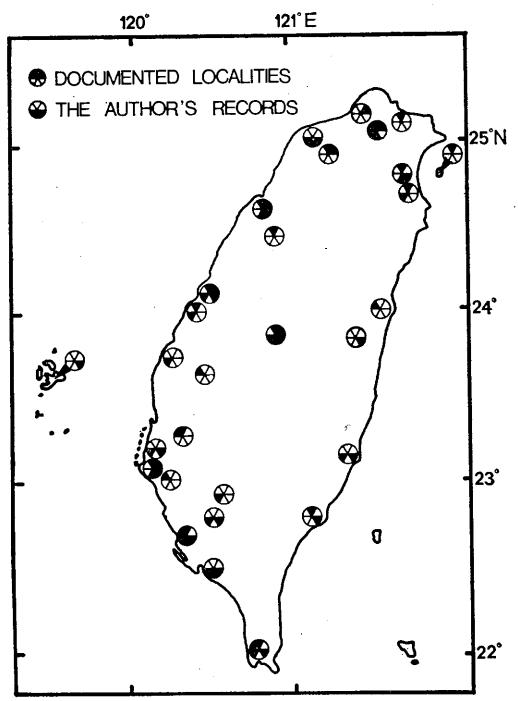

Fig. 4. The distribution map of the Taiwan freshwater mussels.
◐: *Unio douglasiae*
◑: *Cristaria discoidea*
◔: *Anodonta woodiana*

breadth 29-31 mm. The data are similar to those in Table 1 of this paper. The author therefore uses *U. douglasiae* instead of *U. douglasiae taiwanica.*

Cristaria discoidea (Lea, 1834)
(Plate 1)

Diagnosis: Shell height 1.35-1.74, shell breadth 2.47-4.50 in shell length (Table 1); umbo about 1/4-1/3 distance from anterior end (Fig. 1), umbo and postero-dorsal wings flat weakly developed. Each valves with one weakly developed hinge lateral tooth (Plate 2).

Distribution in Taiwan: Keelung City; Taipei City; Shinwu and Chungli, Taoyuan Hsien; Kueishan Island, Ilan and Lotung, Ilan Hsien; Miaoli Hsien; Taichung Hsien; Puyen Village, Changhwa Hsien; Sunmoon Lake, Nantou Hsien; Liuying, Tainan Hsien; Tainan City; Kaohsiung City; Taitung Hsien; Tongkang and Hengchun, Pintung Hsien (Fig. 4).

Anodonta woodiana (Lea, 1834)
(Plate 1)

Diagnosis: Shell height 1.36-1.84, shell breadth 2.05-3.09 in shell length (Table 1); umbo about 1/4 distance from anterior end (Fig. 1), umbo swollen. No trace of teeth in the hinge region of both valves (Plate 2). Scars of the anterior and posterior retractor-pedis incorporated in scars of anterior and posterior respectively (Fig. 1). Both valves with postero-dorsal wings (Plate 2).

Distribution in Taiwan: Taipei City; Tanshui, Taipei Hsien; Shinwu and Chungli, Taoyuan Hsien; Lotung, Ilan Hsien; Miaoli Hsien; Lukang, Changhwa Hsien; Sunmoon Lake, Nantou Hsien; Yunlin Hsien; Penghu Hsien; Chiangchun Stream, Tainan Hsien; Tainan City; Chengkung and Taitung, Taitung Hsien; Meinung, Kaohsiung Hsien; Kaohsiung City; Tongkang and Lungluantan, Pintung Hsien (Fig. 4).

DISCUSSION AND CONCLUSION

The Unionidae of Taiwan has been reported since 1865 (see references above), but no key for species identification was produced until Pace's report in 1975[16]. Unfortunately, the muscle scars, conchomorphometric proportion, electrophoretic patterns and phylogenetic relationship between them are still unclear.

The application of the electrophoretic technique for taxonomical studies of various molluscs shows that protein variation is species specific (Levinton and Koehn[11]; Chang and Wu,[2]). The results of the present investigation show that examination of electrophoretic patterns of soluble muscle proteins is useful to compare closely related unionid species. The numbers of bands and their relative mobility may be considered as criteria to evaluate phyletic relationship; the more closely related species have more bands and relative mobility in common (Tsuyuki, *et al.*)[18]. Comparison of the estimated genetic distance

leads to a conclusion that *U. douglasiae* is well separated from the other two (Figs. 1-2).

As to the conchomorphometric proportion, muscle scars and hinge teeth allow *U. douglasiae* to be easily distinguished from the other two species. The phylogenetic tree in Fig. 3 evidently supports the morphological analysis. Therefore, the Taiwan freshwater mussels can be put into two groups, *U. douglasiae* in one and *A. woodiana* and *C. discoidea* in the other.

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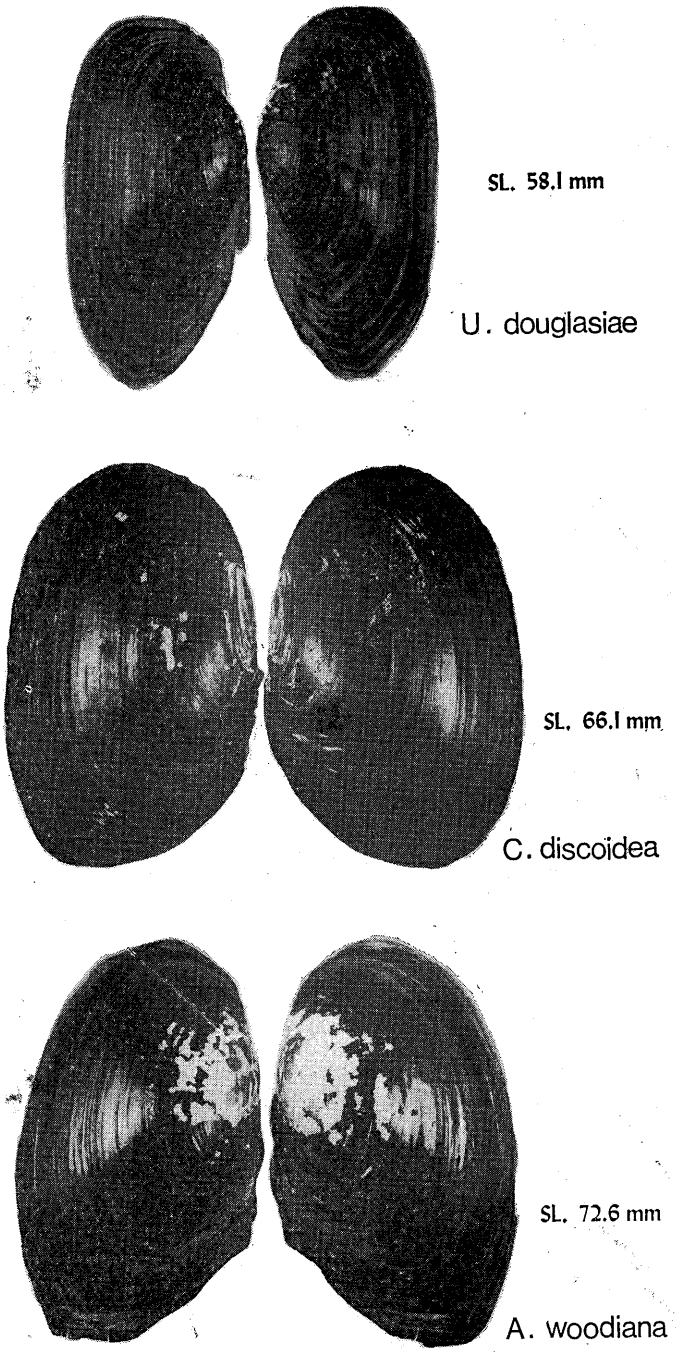

Plate 1. The outer surface of the Taiwan freshwater mussels. The shell length of *U. douglasiae*, *C. discoidea* and *A. woodiana* is 58.1, 66.1 and 72.6 mm respectively.

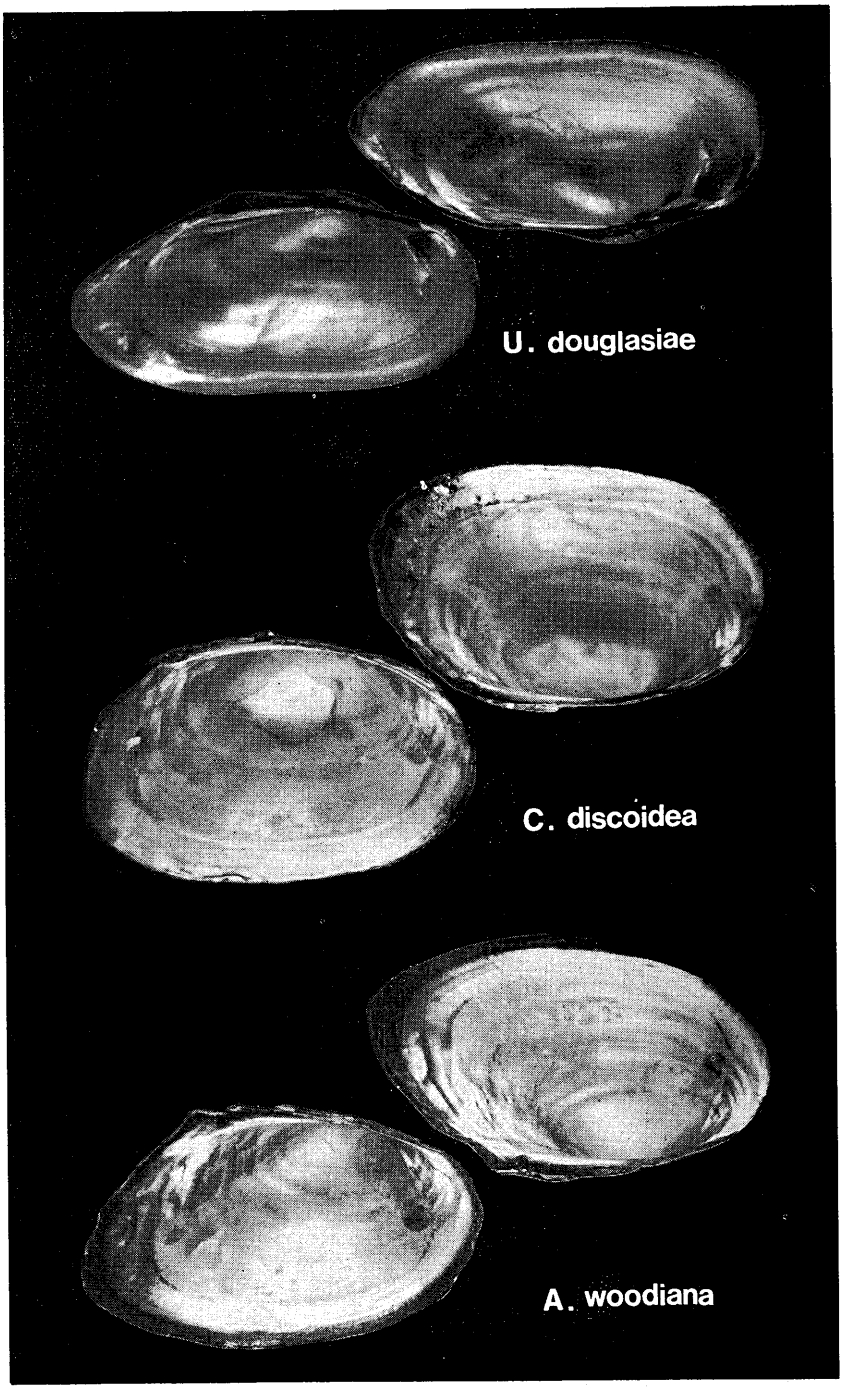

Plate 2. The inner surface of the Taiwan freshwater mussels.

臺灣蚌科雙殼貝系統分類學的研究

巫 文 隆

本文係以形態特徵及水溶性肌肉蛋白質的電泳結果，來比較臺灣蚌科雙殼貝，*Unio douglasiae*, *Anodonta woodiana* 及 *Cristaria discoidea* 的相互關係。*A. woodiana* 和 *C. discoidea* 的前後收足肌痕與閉殼肌痕合併，且爲卵圓形，而 *U. douglasiae* 則互相分離，且呈長隋圓形。*U. douglasiae* 的鉸齒甚爲完整而發達但 *C. discoidea* 者不明顯，在 *A. woodiana* 甚至缺如。從肌蛋白電泳分析結果，可以估算出其遺傳距離 (Genetic distance) 顯示出有種間差異存在。根據上述之結果可以得到本科三種的分類系統樹，可將這三種臺灣產的淡水性雙殼貝分爲兩個種羣：一爲 *U. douglasiae*，另一種羣則包括 *A. woodiana* 和 *C. discoidea*。