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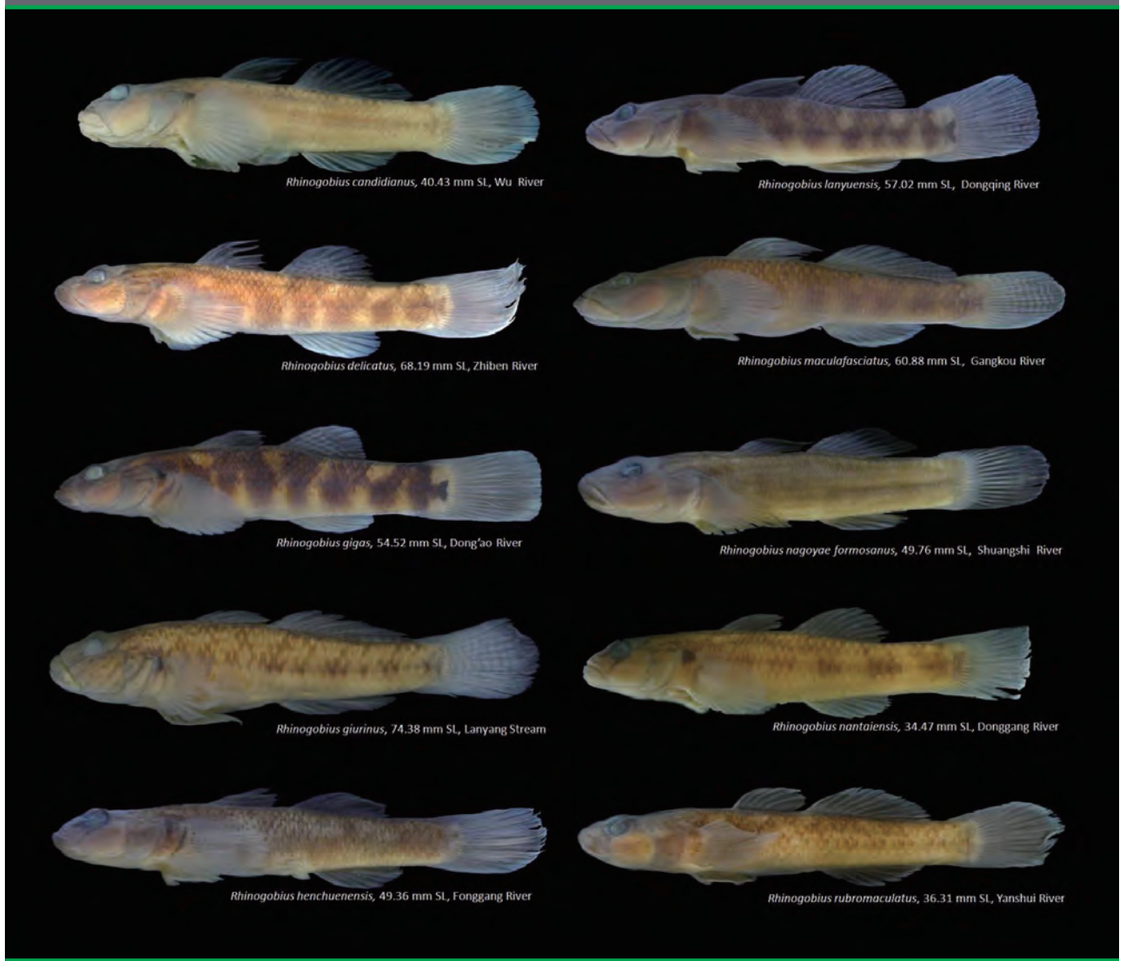

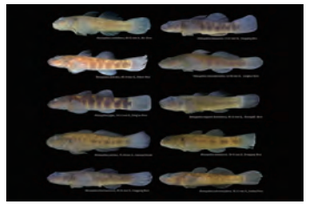

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Morphology and Distribution Patterns of the *Rhinogobius* Species Complexes (Gobiidae) in Taiwan

台灣產吻鰕虎屬魚類的形態與分布模式

Nico Jose Leander[1*], Wann-Nian Tzeng[1,2] and Ming-Fong Yeh[3*]

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Abstract

The morphology and distribution patterns of the *Rhinogobius* species complexes (Gobiidae) in Taiwan were studied based on the specimens collected by Endemic Species Research Institute from various river systems around the island during the period from 1992 to 2005. Morphological examinations revealed ten valid nominal species which can be grouped into two species complexes, *R. giurinus* and *R. brunneus*. The former has only one species while the latter contains nine species namely, *R. candidianus*, *R. delicatus*, *R. gigas*, *R. henchuenensis*, *R. lanyuensis*, *R. maculafasciatus*, *R. nagoyae formosanus*, *R. nantaiensis* and *R. rubromaculatus*. These species can be distinguished from one another by a combination of morphometric measurements, coloration pattern and meristic counts that were slightly different between species and also by their geographical distributions. The shape of the fused pelvic fins

that form the ventral adhesive apparatus was also different between the members of these two species complexes. Members of the *R. brunneus* complex have a round fleshy adhesive apparatus with frenum and connecting membrane while *R. giurinus* has a large, elliptical-shaped adhesive apparatus. In addition, three types of pre-dorsal fin scale pattern were found and can also be used for species identification. Further studies, particularly on habits, habitats, life histories, migratory patterns, and molecular phylogenetic relationships are needed for the conservation and management of this group of gobies.

摘 要

本研究使用行政院農業委員會特有生物研究保育中心於 1992-2005 年間採自全台不同河川之吻鰕虎屬(*Rhinogobius*)魚類樣本，分析其形態特徵與分布模式。在十個有效種樣本中，就其外部形態特徵可分爲極樂吻鰕虎種群 *R. giurinus* complex 與褐吻鰕虎種群 *R. brunneus* complex。極樂吻鰕虎種群在臺灣僅有極樂吻鰕虎(*R. giurinus*)一種，其餘九種均屬褐吻鰕虎種群，包括明潭吻鰕虎(*R. candidianus*)、細斑吻鰕虎(*R. delicatus*)、大吻鰕虎(*R. gigas*)、恆春吻鰕虎(*R. henchuenensis*)、蘭嶼吻鰕虎(*R. lanyuensis*)、斑帶吻鰕虎(*R. maculafasciatus*)、名古屋吻鰕虎(臺灣亞種) (*R. nagoyae formosanus*)、南臺吻鰕虎(*R. nantaiensis*)與短吻紅斑吻鰕虎(*R. rubromaculatus*)。上述物種可藉由測量形質、色斑特徵、計數形質與地理分布稍可辨別其種間差異。兩種群之左、右二枚腹鰭皆已癒合成吸盤狀，但其吸盤形狀在種群間則有不同，褐吻鰕虎種群腹部吸盤形狀呈圓形且有繫帶與連結膜，極樂吻鰕虎種群腹部吸盤較大且形狀呈橢圓形。另外，其背鰭前鱗模式可分爲三型，亦可作爲鑑種的依據。未來，將持續吻鰕虎種群的生態習性、棲地需求、生活史、洄游型態與分子親緣關係之研究，以提供物種保育與經營管理的參考。

Keywords: *Rhinogobius*, species complex, morphology, distribution, Taiwan

關鍵詞：吻鰕虎屬、相似種群、形態、分布、台灣

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Introduction

Gobioidei is one of the largest vertebrate suborders (23% of the Perciformes) with approximately 2,211 extant species classified into 270 genera (Nelson 2006), making it one of the

most speciose and morphologically diverse groups among teleost fishes (Thacker 2003). This group of fish is widely distributed throughout the tropical, subtropical and temperate regions of the world, in freshwater and near shore marine habitats. The high diversity, with small adult size of most goby species has hindered full understanding of their taxa and led to the confusion in their systematic relationship (Wang *et al.* 2001). Also, as compared to other Perciformes, gobies are often morphologically reduced with many species possessing simplifications and losses in various aspects of morphology (Thacker 2003). These factors, coupled with the generally cryptic nature of most goby species and the inherent difficulty to sample them hinder the study of their phylogeny and biology.

Among the Gobioidei, the Asiatic freshwater goby genus *Rhinogobius* Gill 1859, of the family Gobiidae, is of particular interest because of their wide distribution and great variation in coloration, morphology and ecological forms. *Rhinogobius* species are widely distributed on some islands of the West Pacific including Japan (Akihito *et al.* 1993, 2002), Taiwan (Chen and Shao 1996; Chen *et al.* 1998; Chen and Fang 1999), Hainan Province, China, (Chen *at al.* 2002), the Philippines (Herre 1927) as well as continental Asia in Russia, Korea, Vietnam, Laos, Cambodia and Thailand (Kottelat 1989, 2001a, 2001b; Chen and Miller 1998; Chen, Kottelat and Miller 1999; Chen, Wu and Shao 1999; Chen and Kottelat 2000, 2003). Studies on the life histories of this group of gobies indicated that the genus includes both diadromous and non-diadromous species (Mizuno 1960; Mizuno and Goto 1987; Kawanabe and Mizuno 1989; Iguchi and Mizuno 1991; Tzeng and Lin 1996; Akihito *et al.* 1993, 2002; Lee and Chang 1996; Shen *et al.* 1998; Chang *et al.* 2008; Tsunagawa and Arai 2008, 2009). Non-diadromous *Rhinogobius* are mainly landlocked continental species with limited distributions and are usually confined to the upper tributaries of the river systems (Chen and Miller 1998; Chen, Wu and Shao 1999b; Chen, Yang and Chen 1999), while the diadromous types are the migratory species mainly found in many islands with fast flowing rivers and streams.

At present, it is estimated that more than 80 species are known in East and Southeast Asia and some of them are still in need of formal description (Akihito *et al.* 2002; Chen and Kottelat 2003, 2005; Chen and Fang 2006). In Japan, most *Rhinogobius* species reported remain taxonomically uncertain, thus their scientific names has not yet been decided and only common names has been given on the basis of the color of macules and morphological features (Mizuno *et al.* 2001; Tsunagawa and Arai 2009). On the other hand, in Taiwan, two species complexes were recognized, *R. brunneus* and *R. giurinus* (Chen and Yu 1986; Tzeng 1986a). Collections and re-examinations of all *Rhinogobius* specimens from rivers in Taiwan during the last ten years revealed that *R. brunneus* species complex should include at least eight to nine species based on both

morphological and molecular data (mtDNA sequences) as well as on some ecological considerations (Chen 1994; Chen and Shao 1996) while *R. giurinus* species has only a single species. It was reported in previous researches that the members of *R. brunneus* species complex are extremely variable in color and consist of many morphs (Akihito *et al.* 1984; Kawanabe and Mizuno 1989; Masuda *et al.* 1989; Katoh and Nishida 1994; Chen and Shao 1996; Kon and Yoshino 2003). Despite of these findings, however, the *Rhinogobius* species in Taiwan remain taxonomically uncertain and their biological cycle and the parameters leading to such extreme evolution in a small island remain poorly understood. Also, their phylogeny is difficult to resolve using morphological analyses alone because their meristic characters widely overlap, but the specific coloration patterns maybe useful in differentiating the members of the *R. brunneus* species complex (Mizuoka 1974; Masuda *et al.* 1984; Akihito *et al.* 1993; Chen *et al.* 1998).

In the present study, we examined the morphology and the geographical distributions of the members of *Rhinogobius* species complexes in Taiwan based on the fish collection done all over the island from 1992 to 2005. Also, diversity indices such as species richness (S), Margalef's and Menhinick's were calculated and compared between the different regions of Taiwan. S is the most common measurement of biodiversity used by scientists, conservationists and policy makers (Magurran 1988; Gray 2000; Wilsey *et al*., 2005; Flynn *et al*., 2009). It is also the simplest way to describe community and regional diversity. Diversity indices are a necessary tool to calculate and quantify diversity status (van Strien *et al*. 2012). The results obtained here will form the basis for future phylogenetic and ecological studies to help resolve their taxonomy and life histories in Taiwan. Understanding their evolution, distribution, dispersal and life histories is crucial for their management.

Materials and methods

Sample collection

All of the specimens of *Rhinogobius* examined in the present study were collected by Endemic Species Research Institute using electro-fishing from various rivers systems around the island from 1992 to 2005 (Fig. 1 and Table 1). The specimens were fixed with 15% formalin solution and then preserved in 75% ethyl alcohol. All specimens are deposited in the ichthyological collection of the Endemic Species Research Institute in Jiji, Nantou, Taiwan.

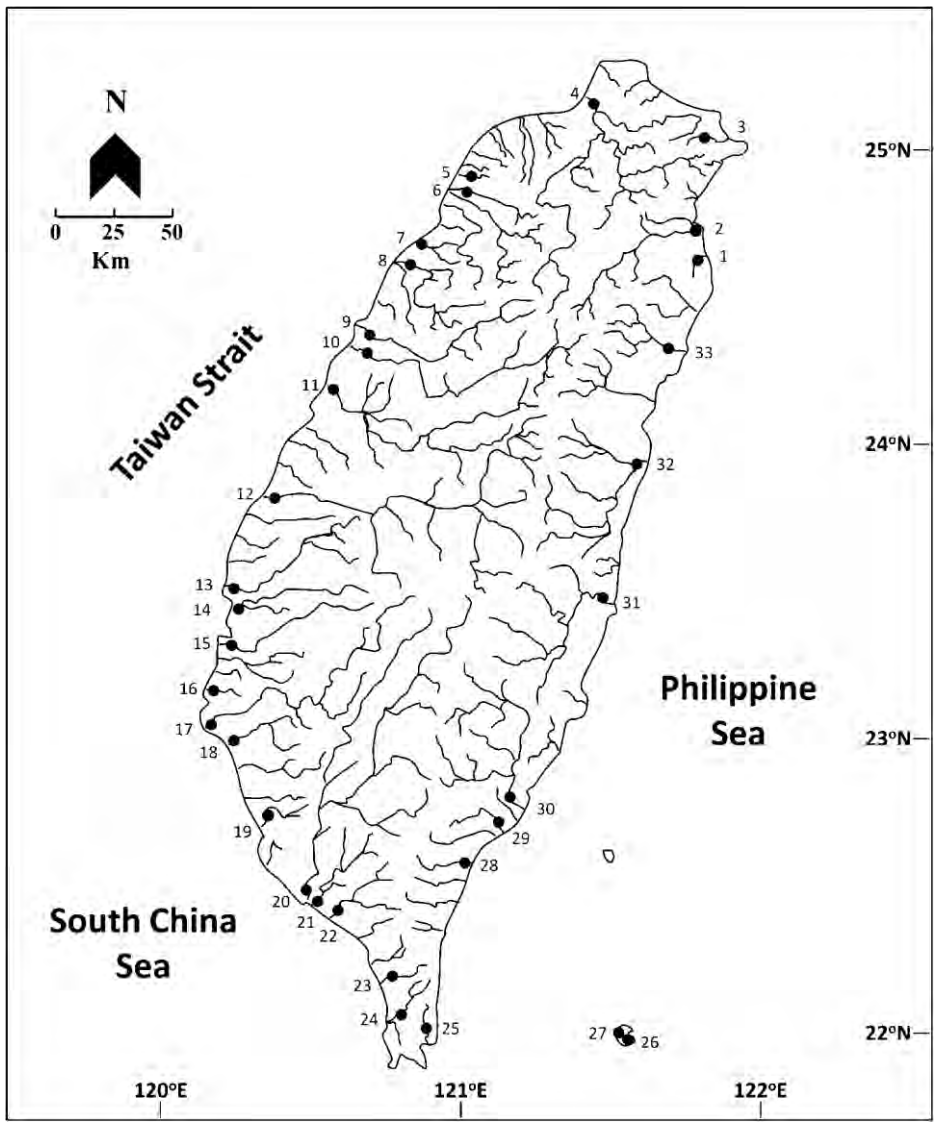

Fig. 1. Map showing the river systems and its tributaries (No. 1-33) around Taiwan where the Rhinogobius species were collected during the period from 1992 to2005. The names of the river systems and its tributaries are shown in Table 1.

Table 1. Data of the *Rhinogobius* species complexes samples collected in Taiwan

Region	No. of sampling location*	River system	Tributary/local branch	Sampling period	Sample size
Northern Taiwan	1	Dong'ao		2004	15
	2	Lan-yang		2003	2
			a. Songluo	2003	3
			b. Annong	2002-2003	25
			c. Cukeng	2002	1
			d. Paigu	2003	4
			e. Duowang	2004	1
			f. Xiao	2002	11
			g. Sichong	n/a	1
			h. Han	2003	6
			i. Malun	1999	106
	3	Shuangshi		2003	11
			a. Dingzailan	2002, 2004	15
			b. Hou Fanzaikeng	2002-2004	14
			c. Mudan Stream	2004	9
	4	Tanshui			
			a. Huiyao	2000	11
			b. Keelung	2000	17
			c. Dahan	1995, 2000, 2003	62
			d. Xindian	2002, 2004	42
	5	Fengshan		2000	17
	6	Touqian		2000	5
			a. Youluo	2000	10
Central Taiwan	7	Zhonggang		2000	4
			a. Dadong	2000	1
			b. Emei	2000	5
	8	Houlong		2000, 2005	54
			a. Wenshui	2000	6
	9	Da'an		2001	4
			a. Wushikeng	1997	1
	10	Dajia		1999, 2001, 2003	22
	11	Wu		2000, 2003-2005	250

			a. Fazi	2001-2002, 2005, 2007	85
			b. Mei	2004	41
			c. Maopukeng	2004	16
			d. Toubiankeng	2004-2005	7
			e. Dongbiankeng	2004	10
			f. Caohu	2005	1
			g. Cukeng	2005	4
			h. Zhangping	2005	31
			i. Wupengkeng	2005	6
			j. Maoluo	2005	47
			k. Dali	2005	32
			l. Pinglin	2005	38
			m. Nangang	2004	70
			n. Sanguang	2004	7
			o. Meiyuan	2005	3
			p. Beikeng	2004	17
			q. Gan	2005	6
			r. Shigang	2004	6
	12	Zhuoshui		2001	12
			a. Qingshui	2001, 2003	50
			b. Dongpurui	2003	15
			c. Pinglai	2004	3
	13	Beigang		2004-2005	59
			a. Huaxing	2001	2
	14	Puzi		2001	8
			a. Shitou	2001	1
	15	Bazhang		2002	14
			a. Chilan	2000-2002	30
	16	Jishui		2002	1
Southern			a. Baishui	2002	9
Taiwan	17	Zengwen		2002-2004	30
			a. Houjue	2002-2003	46
			b. Cailiao	2003	12
			c. Cao	2002-2003	106
	18	Yanshui	a. Xu	2002	4
			b. Wukan	2002	5

	19	Angongdian		2002	1
	20	Kaoping		2000-2003	70
			a. Qishan	2002-2004	29
			b. Laonong	2002, 2004	8
			c. Qingshui	2002-2003	95
			d. Koushe	2002	15
	21	Donggang		2000-2003	7
	22	Linbian		2002	4
			a. Walusi	2002	2
	23	Fenggang		2004	3
			a. Shuangliu	2002-2004	18
			b. Daren	2004	8
	24	Sichong		2002	39
			a. Damei	2002	4
			b. Mudan Stream	2002	38
	25	Gangkou		2004	8
			a. Jiadoulu	2004	3
	26	Dongqing		2004	1
	27	Yeyou		2004	1
Eastern Taiwan	28	Nantaimali		2000	4
	29	Zhiben		2004	52
	30	Beinan			
			a. Luye	2000	3
	31	Hsiukuluan		2002-2003, 2005	41
			a. Hongye	1992, 2003-2004	18
			b. Lehe	2003	6
			c. Fuyuan	2003	2
			d. Amei	2003	8
	32	Hualien			
			a. Lao Stream	2001-2004	36
			b. Bai-bao Stream	2002-2004	65
			c. Matai'an	2001-2002	4
	33	Heping		2003	26
Total					2188

*No. of sampling location refer to Fig.1

Morphometric measurements and species identification

After initial species identification, 20 individuals (when applicable) were chosen from each species for morphometric measurements and meristic counts following the methods of Akihito *et al.* 1984, Chen and Shao 1996 and Lee and Chang 1996. All measurements and counts were made from preserved specimens. Morphometric measurements include total length, standard length, head length (HL), body depth (BD) and width (BW), pre-dorsal fin (PDL) and pre-anal fin (PAL) lengths, caudal peduncle length (CPL) and depth (CPD) and 1st dorsal fin (D1bl), 2nd dorsal fin (D2bl) and anal fin (Abl) base lengths. Meristic counts on the other hand, include 1st dorsal fin (D1), 2nd dorsal fin (D2), pectoral fin (P1) and anal fin (A) ray counts.

Species diversity index calculation

The species diversity per geographic region was calculated using different species diversity indices (Equations 1-2):

(1) Margalef's species diversity index,

$$D = \frac{(S-1)}{\ln N}$$

(2) Menhinick's species diversity index,

$$D = \frac{S}{\sqrt{N}}$$

where D is the number of species for a given number of individuals, S is the number of species recorded (species richness) and N is the number of individuals (Jennings *et al*. 2001). The higher the index, the greater the diversity.

Results and discussions

Species Composition

A total of 2,188 individuals were collected from 33 river systems around Taiwan during the period from 1992 to 2005, and out of these, ten species were identified (Fig. 2) and assigned into two species complexes, *R. giurinus* and *R. brunneus*. *Rhinogobius giurinus* complex has only one species under it, *R. giurinus* (Fig. 2d), while *R. brunneus* complex contains the remaining nine species namely *R. candidianus* (Fig. 2a), *R. delicatus* (Fig. 2b), *R. gigas* (Fig. 2c), *R. henchuenensis* (Fig. 2e), *R. lanyuensis* (Fig. 2f), *R. maculafasciatus* (Fig. 2g), *R. nagoyae formosanus* (Fig. 2h), *R. nantaiensis* (Fig. 2i) and *R. rubromaculatus* (Fig. 2j). About 95.34% of the specimens examined were adults while the rest of the collection was composed of juveniles (4.43%) and larvae (0.23%) (Table 2).

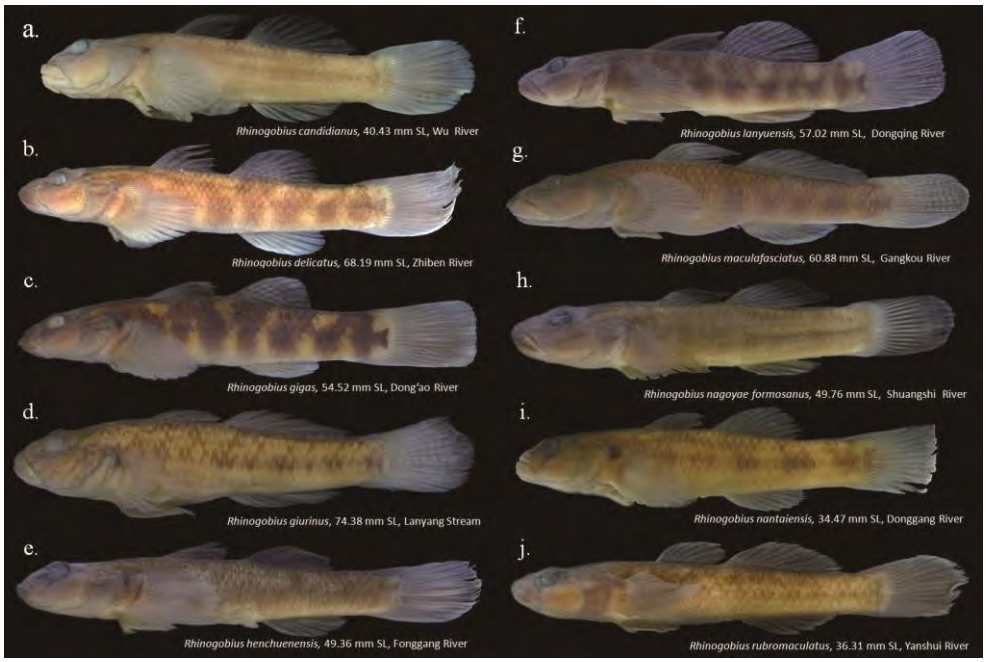

Fig. 2. External features of the 10 *Rhinogobius* species found in Taiwan.

Table 2. Composition of developmental stages and comparison of the 3 morphological characteristics (Pre-dorsal fin scale pattern, and ventral adhesive apparatus and scale types) of the 10 *Rhinogobius* species collected in Taiwan

Species	Stage composition			Total	Pre-dorsal fin scale pattern[1]	Ventral adhesive apparatus type[2]	Scale type
	Larva	Juvenile	Adult				
R. candidianus	5	62	1126	1193	Type 2	Type 2	ctenoid (flank) and cycloid (predorsal area)
R. delicatus	0	1	94	95	Type 3	Type 2	
R. gigas	0	0	183	183	Type 3	Type 2	
R. giurinus	0	9	68	77	Type 1	Type 1	ctenoid (flank and predorsal)
R. henchuenensis	0	2	112	114	Type 1	Type 2	
R. lanyuensis	0	0	2	2	Type 3	Type 2	
R. maculafasciatus	0	3	311	314	Type 2	Type 2	ctenoid (flank) and cycloid (predorsal area)
R. nagoyae formosanus	0	0	6	6	Type 3	Type 2	
R. nantaiensis	0	0	34	34	Type 2	Type 2	
R. rubromaculatus	0	20	150	170	Type 3	Type 2	
Total	5	97	2,086	2,188			

[1]Predorsal fin scale pattern refer to Fig. 3; [2]Ventral adhesive apparatus type refer to Fig. 5

Morphological Features

Ventral adhesive apparatus

The shape of the fused pelvic fins that forms the ventral adhesive apparatus was different between the members of these two *Rhinogobius* species complexes (Fig. 3 and Table 2). *Rhinogobius giurinus* has a large, elliptical-shaped adhesive apparatus (Type 1) while the members of the *R. brunneus* species complex have a round adhesive apparatus with frenum and connecting membrane (Type 2). Also the distance of the tip (posterior part) of the ventral adhesive apparatus in relation to the anus and genital pore was different between the members of these two species complexes. When adpress the posterior of part of the Type 1 ventral adhesive apparatus is closer to the anal pore as compared to that of Type 2.

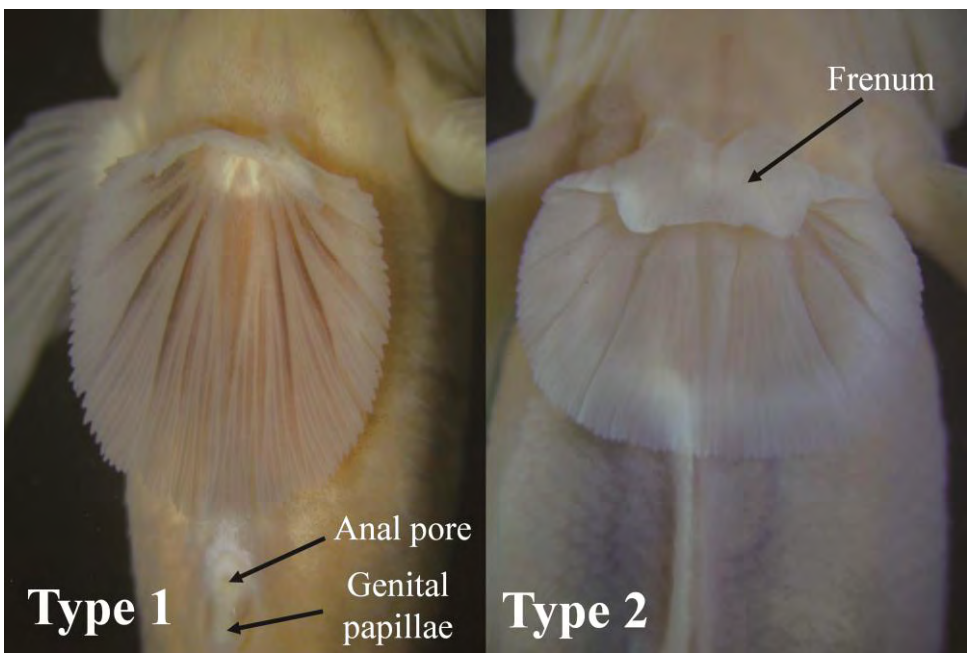

Fig. 3. Two types of fused pelvic fins that form the ventral adhesive apparatus in *Rhinogobius* spp. Type 1: *R. giurinus* complex; Type 2: *R. brunneus* complex.

Body scale type

Scales were collected along the flank, head and caudal regions of each species and examined under the microscope. The scales of the *Rhinogobius* species examined were classified as ctenoid (Fig. 4) and cycloid. Ctenoid was the only type of scale observed in the different body regions of *R. giurinus*. On the other hand, all the members of the *R. brunneus* complex have two types of scales: ctenoid on the flank region and small cycloid scales on the predorsal fin area. Ctenoid scale can be divided into two fields, the anterior field and the posterior field. The anterior field is the part of the scale that is buried

(embedded) in the fish skin. Prominent vertical grooves that begin outward until the scale margin can be observed in the anterior field, and these are called secondary radii (Fig. 4b). In between these secondary radii (inter-radial area) and also in the lateral field of the scale were elevated ridges called circuli that usually appear as lines that more or less follow the outline of the scale (Fig. 4b). The posterior field of the scale, on the other hand, is the exposed part and it contains pigment cells (chromatophores) (Fig. 4c); and the tiny, comb-like projections on the margin called ctenii (Fig. 4d).

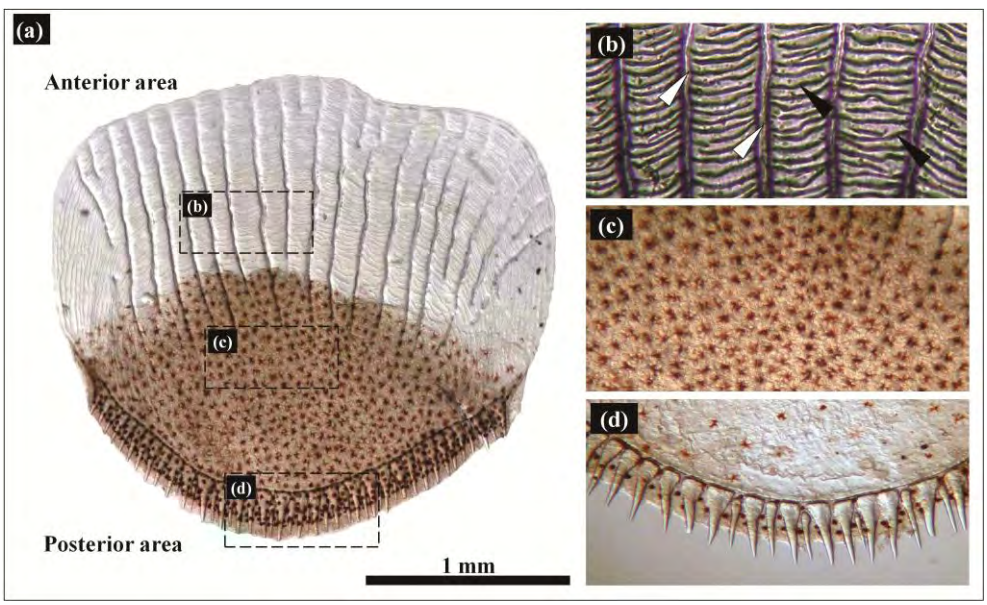

Fig. 4. Microstructures of the scale of *Rhinogobius*. (b-d) is partially magnified from the scale (a): (b) circuli (black arrow) and radii (white arrow), (c) pigment cells (chromatophores) and (d) ctenii.

Pre-dorsal fin scale pattern

Three distinct types of pre-dorsal fin scale pattern were found in the ten *Rhinogobius* species examined (Fig. 5). In first type of pre-dorsal fin scale pattern, the scaled area extends almost to the posterior margin of the eyes (Fig. 5a). *Rhinogobius giurinus* and *R. henchuenensis* exhibited this type of pattern (Table 2). On the other hand, in the second and the third type of pre-dorsal fin scale patterns, the scaled area does not extend beyond the posterior margin of the preoperculum with the third type noticeably shorter in coverage (Fig. 5c) than the second type (Fig. 5b). *Rhinogobius candidianus, R. maculafasciatus* and *R. nantaiensis* exhibited the second type pattern while *R. delicatus, R. gigas, R. lanyuensis, R. nagoyae formosanus* and *R. rubromaculatus* exhibited the third type.

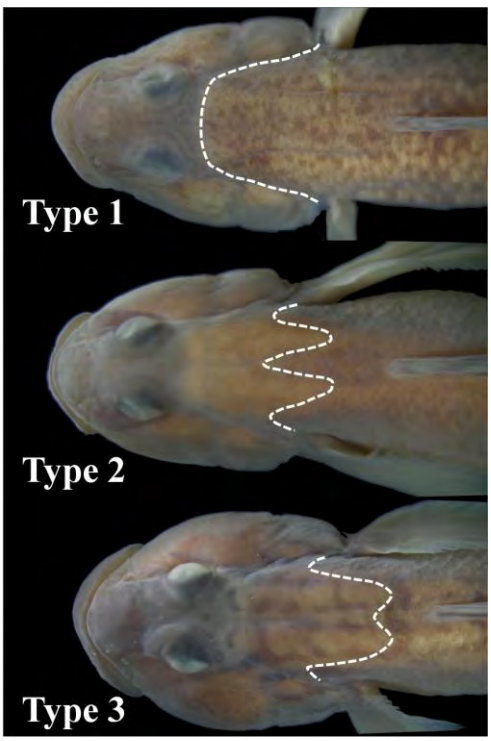

Fig. 5. Types of pre-dorsal fin scale patterns found among different *Rhinogobius* species from Taiwan.

Morphological measurements and fin ray counts

Morphometric measurement data and meristic counts are listed in Table 3. *Rhinogobius lanyuensis* has the shallowest BD (15.12±1.29 mm) while *R. maculafasciatus* has the deepest (23.89±2.17 mm). In terms of BW, *R. gigas* has the narrowest body (15.88±1.89 mm) while *R. maculafasciatus* has the widest (20.33±2.12 mm). PDL ranged from 39.58±1.74 mm in *R. rubromaculatus* to 42.90±1.51 mm in *R. lanyuensis* while PAL ranged from 62.57±1.26 mm in *R. giurinus* to 67.32±1.73 mm in *R. nagoyae formosanus*. CPL ranged 16.22±2.30 mm in *R. rubromaculatus* to 19.77±0.84 mm in *R. lanyuensis*. CPD on the other hand, ranged from 12.39±0.96 mm in *R. giurinus* to 14.75±1.49 mm in *R. henchuenensis*. D1bl ranged from 15.83±1.64 in *R. henchuenensis* to 18.30±1.33 mm in *R. candidianus* while D2bl ranged from 16.34±1.39 mm in *R. gigas* to 20.80±1.64 mm in *R. rubromaculatus*. Abl ranged from 12.80±1.33 in *R. delicatus* to 16.20±1.17 mm in *R. rubromaculatus*. *Rhinogobius maculafasciatus* has the longest head length (34.06±1.87 mm) while *R. nantaiensis* had the shortest head (30.50±2.15 mm). The D1 of the specimens examined ranged from 5 to 6 while D2 from 7 to 10. A on the other hand ranged from 7 to 10 while P1 ranged from 15 to 22.

The members of the *Rhinogobius* species complex of Taiwan have only very few distinguishable morphological differences except probably for their color patterns. Chen and Shao (1996) also indicated that the *R. brunneus* species complex in Taiwan has radiated into many distinct morphological species with a high degree of local endemism. But previous researches have indicated that these species exhibit distinct segregation in terms of the ecological niche they occupy such as preference for fast or slow water current, upstream or downstream areas, deep or shallow waters, migratory habit and egg size diversity (Chen and Shao 1996, Lee and Chang 1996, Shen *et al*. 1998, Cheng *et al*. 2005a). Therefore, it is important to use a combination of morphological characters and ecological considerations in identifying the different *Rhinogobius* species in Taiwan.

Table 3. Comparison of meristic counts and morphometric measurements (mean ± S.D.) among 10 different *Rhinogobius* species in Taiwan. All values shown except for the meristic counts are percentages (%) as ratio with standard length

	R. candidianus	***R. delicatus***	***R. gigas***	***R. giurinus***	***R. henchuenensis***	***R. lanyuensis***	***R. maculafasciatus***	***R. nagoyae formosanus***	***R. nantaiensis***	***R. rubromaculatus***
Sample size	20	20	20	20	20	2	20	6	20	20
Morphometric										
BD	20.69±2.47	20.33±3.52	17.19±1.78	20.38±2.83	21.90±3.13	15.12±1.29	23.89±2.17	19.19±1.55	20.94±3.49	19.94±2.89
BW	19.21±2.40	18.37±2.52	15.88±1.89	17.23±1.85	18.81±1.66	16.44±3.57	20.33±2.12	17.02±2.68	18.67±2.77	18.09±2.36
PDL	40.93±2.19	41.92±1.64	41.38±1.99	40.21±1.12	42.77±1.31	42.90±1.51	41.75±4.74	42.06±1.53	42.28±4.21	39.58±1.74
PAL	64.17±2.33	65.84±2.30	66.90±3.78	62.57±1.26	66.92±1.82	65.34±2.11	64.53±1.58	67.32±1.73	63.01±2.17	63.81±1.43
CPL	18.68±1.79	18.70±1.93	19.42±1.55	19.35±1.31	17.32±1.77	19.77±0.84	18.67±1.52	17.80±0.83	19.36±2.18	16.22±2.30
CPD	14.36±0.80	13.76±1.41	13.02±0.83	12.39±0.96	14.75±1.49	14.35±0.42	13.19±0.67	13.33±0.51	13.76±0.80	13.60±0.78
D1bl	18.30±1.33	17.54±1.41	16.82±1.17	18.02±1.66	15.83±1.64	16.58±0.62	18.22±1.98	16.01±0.75	15.93±2.31	18.04±1.86
D2bl	18.58±1.02	16.38±1.67	16.34±1.39	19.07±1.14	17.56±0.85	16.92±0.56	17.06±0.99	16.45±1.34	17.81±1.08	20.80±1.64
Abl	14.07±1.40	12.80±1.33	13.09±1.40	15.90±0.86	14.23±1.40	13.01±0.16	13.30±1.02	13.84±1.40	13.33±0.93	16.20±1.17
HL	30.88±3.40	31.02±1.91	31.56±3.09	32.53±1.24	33.72±2.15	33.98±0.72	34.06±1.87	34.71±3.34	30.50±2.15	31.57±1.42
Meristic										
D1	5-6	6	6	6	6	6	6	6	6	6
D2	7-10	5-6	8-10	10	9-10	9-10	9-10	8-9	10	9-10
A	7-10	9-10	8-10	10	9-10	10	9-10	8-9	10	8-10
P1	15-18	20-22	21-22	20	18-21	20	18-21	17-18	19-20	15-18

Geographical Distribution

Thirteen years of intensive collections from various river systems and its drainages around Taiwan revealed that the different *Rhinogobius* species examined exhibited species-specific distribution patterns (Fig.6). Some species have a very narrow geographic distribution while the others are distributed widely. Among all the species examined, *R. candidianus* and *R. giurinus* can be found in the majority of the river systems in Taiwan (Fig. 6a and 6d). *Rhinogobius delicatus*, on the other hand, can only be found in rivers along the northern, eastern and southern Taiwan (Fig. 6b). *Rhinogobius henchuenensis* and *R. maculafasciatus* can only be found in the rivers in southern Taiwan (Fig. 6e and 6g), while *R. lanyuensis* seems to be restricted only in Orchid Island (Lanyu), a 42 km^2 volcanic island off the southern coast of Taiwan (Fig. 6f). *Rhinogobius nagoyae formosanus* is only restricted in northern Taiwan (Fig. 6h) and *R. nantaiensis* can only be found in the rivers in central and southern Taiwan (Fig. 6i). *Rhinogobius rubromaculatus* is found in northern, central and southern Taiwan (Fig. 6j). In addition, population genetic studies on some of the species such as *R. maculafasciatus* and *R. rubromaculatus* showed that there is little genetic differences among their respective populations in Taiwan (Cheng *et al*. 2005a,b), indicating contemporary gene flow among its populations through oceanic larval dispersal. Unfortunately, the population genetic structures of the other *Rhinogobius* species in Taiwan have not yet been investigated. On the other hand, the geographic distribution pattern of the different *Rhinogobius* species in Taiwan might be affected by a number of factors such as temperature preference, river topology, inter-specific competition and habits of different goby species. The dominance of one or more species in a certain region might restrict the ability of the other species to colonize that area. Species that prefers warmer water temperature might not be able to colonize the colder northern region of Taiwan. But some researchers have suggested that the occurrence of freshwater fish species of marine origin (like amphidromous gobies) in northern Taiwan is probably because of the strong Kuroshio Current that flows from south to north (Chen and Fang 1999). Diadromous types usually have wider geographic distribution since their oceanic larval duration allows them to be transported in areas not reached by non-diadromous types. Non-diadromous types also have limited opportunities for gene flow (Tzeng *et al*. 2006). Also, since each region in Taiwan offer different types of habitat, it is expected that the *Rhinogobius* species in the island will have a variety of ecological forms according to the niche they occupy. Species that prefers muddy or sandy river substrate with slow river flow were often found in western Taiwan while those that preferred rocky substrate, steep river topology and swift river current were found in eastern Taiwan.

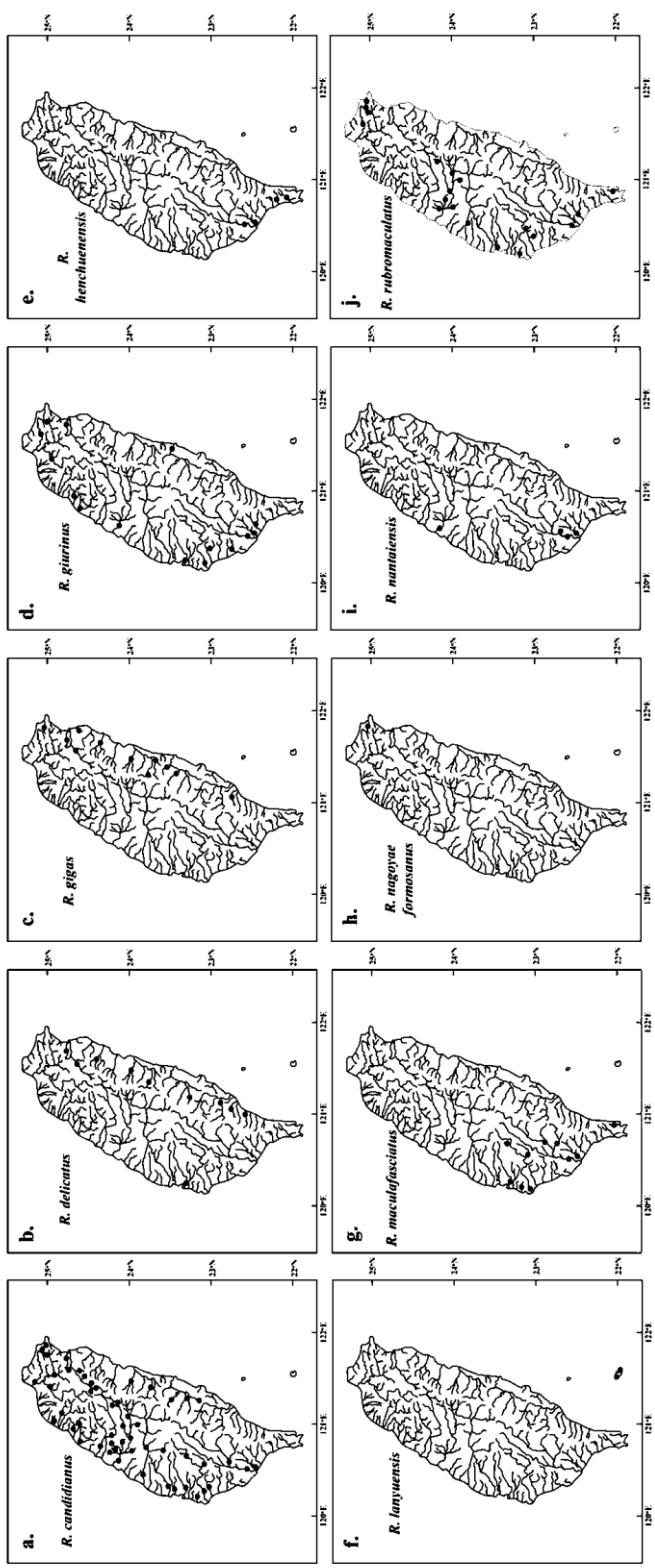

Fig. 6. Different distribution patterns of the 10 *Rhinogobius* species in Taiwan.

Species diversity

In terms of the number of species observed, southern Taiwan is the most speciose region in the island with seven out of ten *Rhinogobius* species found, followed by northern Taiwan with six species (Table 4). Central and eastern Taiwan has similar number of species, four each. The other indices of species diversity showed different pattern. Margalef's diversity index indicated that southern Taiwan was the most speciose region while central Taiwan was the least. Menhinick's on the other hand, indicated that northern region of Taiwan was the most speciose while its central region was the least. High species diversity is also expected in southern Taiwan since it is a subtropical region and productivity is high. On the other hand, the restriction of some freshwater fish species to the western or eastern part of Taiwan was thought to be highly correlated with the topographical isolation due to the rise of the Central Mountain Range about one million years ago (Lin 1966; Tzeng 1986b; Wang *et al*. 1999, 2000). Taiwan is an isolated island located between East and Northeast China and South China Sea in the southwest and is under the influence of subtropical weather regimes. The waters on its western slope are shallow while very deep (up to 1,000-2,000 m) on the eastern slope (Wu *et al*. 2007). On the other hand, the majority of the river systems on the island mainly flows to the western and eastern coasts and is well separated by the Central Mountain Ridge (Page and Suppe 1981; Wu *et al*. 2007). These features resulted in a unique freshwater fish fauna on the island. Therefore the study of freshwater fishes in Taiwan is extremely important because it confirms the relationship between the island and its adjacent areas. Tzeng *et al*. (2006) also stressed that the use of freshwater species for biogeography studies are very appropriate primarily because of their restricted distribution and strong association between evolutionary history and geological events.

Table 4. Comparison of species diversity indices of *Rhinogobius* species among different regions of Taiwan

Region	No. of specimen collected	Species diversity indices		
		Species richness	Margalef's	Menhinick's
North	388	6	0.84	0.30
Central	915	4	0.44	0.13
South	620	7	0.93	0.28
East	265	4	0.54	0.25

Conclusion and perspectives

The *Rhinogobius* species in Taiwan exhibits great variations in coloration, morphology and ecological forms and such variations resulted in confused and problematic taxonomy. A reliable phylogeny for the group would help in setting evolutionary hypothesis that resulted in their extreme diversity in such a small island like Taiwan. Also, determining their phylogeny would establish their exact relationship and would help establish a timeline for their speciation. This would present a more detailed evolutionary history of the diverse *Rhinogobius* species in the island. It is also interesting to explore the effect of different barriers to dispersal such as mountain ranges and oceanographic current systems on the distribution pattern of the different *Rhinogobius* species. Understanding this information is essential for their conservation and management and also will present an important contribution to the understanding of the historical biogeography of freshwater fishes of East Asia.

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台 9 線蘇花公路沿線猛禽資源調查

Species and abundance survey of raptors along Suhua Highway

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摘　要

自 2012 年 3 月至 2013 年 2 月於台 9 線蘇花公路蘇澳至大清水段沿線進行猛禽群聚調查。猛禽群聚共記錄到 3 科 11 種日行性猛禽及 1 科 2 種夜行性猛禽。日行性猛禽中有 4 種爲留鳥，分別是林鵰(*Ictinaetus malayensis*)、蛇鵰(*Spilornis cheela hoya*)、鳳頭蒼鷹(*Accipiter trivirgatus*)及台灣松雀鷹(*A. virgatus fuscipectus*)；6 種爲過境鳥或候鳥，分別是赤腹鷹(*A. soloensis*)、灰面鵟鷹(*Butastur indicus*)、鵟(*Buteo buteo*)、魚鷹(*Pandion haliaetus*)、遊隼(*Falco peregrinus*)及紅隼(*F. tinnunculus*)。另外一種東方蜂鷹(*Pernis ptilohynchus*)，則可能有留鳥或候鳥 2 種型態。2 種夜行性猛禽皆爲留鳥，分別是領角鴞(*Otus lettia*)及黃嘴角鴞(*O. spilocephalus*)。蛇鵰爲日行性留鳥猛禽中最普遍的物種，在 9 個猛禽調查樣點中皆可發現。9 個研究樣點中以觀音及漢本樣點的猛禽物種多樣性 9 種最高。以猛禽在各季節的多樣性而言，秋季猛禽的物種多樣性 11 種最高。

Abstract

We investigated species and abundance of raptors along Suhua Highway from March 2012 to February 2013. Eleven species of diurnal raptors and two nocturnal raptors were recorded from nine study sites along Suhua Highway. There were four resident species, specifically the Indian Black Eagle

(*Ictinaetus malayensis*), Crested Serpent Eagle (*Spilornis cheela hoya*), Crested Goshawk (*Accipiter trivirgatus*) and Besra (*A. virgatus fuscipectus*); and six migration species, specifically the Chinese Goshawk (*Accipiter soloensis*), Grey-faced Buzzard (*Butastur indicus*), Common Buzzard (*Buteo buteo*), Osprey (*Pandion haliaetus*), Peregrine Falcon (*Falco peregrinus*) and Common Kestrel (*F. tinnunculus*). The Oriental Honey Buzzard (*Pernis ptilohynchus*) may be considered as either a resident species or migration species. The two nocturnal raptors are both resident species, specifically the Collared Scops Owl (*Otus lettia*) and Mountain Scops Owl (*O. spilocephalus*). The Crested Serpent Eagle is the most common diurnal raptor, as it was recorded in all study sites. The diversity of raptor at Guanyin site and Hanben site were the highest among all of the sites. In terms of seasonal changes, raptor diversity was high during autumn.

關鍵字：猛禽、台 9 線蘇花公路

Key words: raptors, Suhua Highway

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緒　言

族群監測在保育生物學上扮演重要的角色，在特定保育問題的早期階段和建議可能方案上，可提供充分的資訊。鳥類群聚的多樣性及豐富度(族群大小或密度)被廣泛運用於鳥類保育，用於監測或評估該地區鳥類群聚的改變，是否受到生態環境破壞影響的具體情況，及量化退化與回復的過程(Padoa-Schioppa *et al.* 2006, Buckland *et al.* 2008)。鳥類常被視爲一個地區的生物指標(bioindicator)，其主要原因爲：(1)鳥類生態已被廣泛研究；(2)鳥類與棲地間的關係非常緊密；(3)牠們的營養階層較廣泛，大多位於食物金字塔的上層；(4)牠們容易觀察(邱等 2001, Padoa-Schioppa *et al.* 2006)。因此，有許多國家或地區以鳥類作爲長期監測或評估環境的工具，如美國奧杜邦學會(Audubon Society)舉辦的聖誕節鳥類調查(Christmas Bird Count, CBC；Temple and Wiens 1989)及北美洲繁殖鳥類調查(Breeding Bird Survey, BBS；Robbins *et al.* 1989)，記錄著北美洲鳥類群聚趨勢隨環境變遷的改變。

猛禽(Raptor)在生態系中扮演重要的角色，位於食物金字塔較高位階，以其它動物爲主食，且猛禽對於環境的變動敏感，可作爲監測環境變動之指標物種。近年來由於猛禽的棲地快速地消失、破碎化、單一化及汙染，加上人爲濫捕，使得猛禽的族群數量受到威脅(Alvarez-Lopez and Kattan 1995, Bildstein *et al.* 1998)。藉由監測某些猛禽的族群動態趨勢，能

提早發現棲地遭受破壞的警訊。因此對於猛禽資料的收集有迫切需求，以設計因應猛禽族群對環境變遷的長期監測。

台9線蘇花公路目前針對猛禽資源的調查研究有限，大部分猛禽紀錄來自動物資源、鳥類資源調查或環境影響評估報告(周 1993；財團法人中華顧問工程公司 1994；和中電力股份有限公司 1996；楊 2002；毛及陳 2009；弘益生態有限公司 2010)。由於猛禽的族群密度低和活動範圍大等特性，使得物種豐富度受調查努力量影響，且調查方法與燕雀目(Passeriformes)鳥類不同，因此一般動物資源報告很難呈現該區域之猛禽組成。本研究目的希望藉由猛禽資源調查，瞭解蘇花公路沿線的猛禽組成及季節性的分布，提供作爲保育措施之參考。

材料與方法

日行性猛禽調查通常利用猛禽隨氣流飛翔時進行觀察，因此於台9線蘇花公路蘇澳至大清水段沿線選擇9個具有視野良好、植被環境佳等條件之固定調查樣點(圖1；附錄1)。蘇澳日月宮樣點周遭棲地在有人爲干擾之淺山地區以先鋒林和次生林爲主，蘇澳港附近山區則保有天然闊葉林環境。永樂樣點周遭棲地以次生林爲主，部分山區仍保有天然闊葉林環境。東澳樣點周遭棲地以次生林爲主，部分山區仍保有天然闊葉林環境。武塔樣點周遭棲地以次生林爲主，部分山區仍保有天然闊葉林環境。觀音樣點周遭棲地因山勢陡峭人爲干擾較少，棲地組成以天然闊葉林爲主。漢本樣點，蘇花公路西側部分屬於天然闊葉林環境，東側則是漢本新生地，棲地由雜木林和草生地組成。和中樣點周遭棲地，地勢平緩處以銀合歡、山芙蓉、構樹、血桐等植物爲主，山勢陡峭的部分仍保有天然闊葉林環境。和仁樣點周遭棲地，地勢平緩處以次生林爲主，地勢陡峭的山區仍保有天然闊葉林環境。大清水樣點因山勢陡峭人爲干擾較少，棲地組成以天然闊葉林爲主。

猛禽可分爲日行性猛禽(Diurnal raptor)及夜行性猛禽(Nocturnal raptor)，因生態習性不同調查方法亦不同。日行性猛禽在分類學上屬隼形目(Falconiformes)猛禽，採定點計數法(Point counts)調查，每次調查均挑選晴天進行。由於猛禽活動範圍大(Home range)，有時會從鄰近地區飛入調查範圍，有時則飛出調查範圍外。如果在一個調查樣點停留過久，可能會有重複計數的問題(Fuller and Mosher 1987)。因此調查時間選擇在早上 8 點至 11 點及下午 2 點至 5 點，猛禽活動頻繁時段進行調查，且同一調查點早上和下午的調查須爲不同調查日期，且每季每次調查間隔 1 個月，以降低重複計數的問題。調查主要以 ZEIZZ 10×40 的雙筒望遠鏡觀察，必要時並以單眼相機進行個體拍攝記錄。觀察記錄的重點包括種類、出現數量、出現方位、飛行方式、成幼、色型等，如遇繁殖期或覓食行爲時，會額外記錄其行爲重點或覓食的種類與位置等。

回播法(Playback)通常用於夜行性猛禽、隱蔽性高或不易觀察的鳥種(Bibby *et al.* 2000)。鴞形目(Strigiformes)猛禽大多於傍晚至清晨間活動，不易觀察，因此利用貓頭鷹具領域性的特性，以回播法播放同種或異種貓頭鷹的鳴叫，進而誘發棲息該區域的貓頭鷹回應，以提升對貓頭鷹的察覺度。本研究調查時間從晚上 7 時至 10 時，依當日的天候狀況進行調整，且每次樣點間調查順序互換。調查方法是到達樣點後先聆聽 1 分鐘，之後每種貓頭鷹的聲音依

其體型大小，回播 1 分鐘，然後再聆聽 3 分鐘爲 1 循環(Cornulier and Bretagnolle 2006)。調查時所播放的聲音依貓頭鷹體型大小依序爲：鵂鶹 (*Glaucidium brodiei*) 、黃嘴角鴞 (*Otus spilocephalus*)、領角鴞(*O. lettia*)及褐鷹鴞(*Ninox scutulata*)。察覺貓頭鷹鳴叫時亦記錄鳴叫個體之方位，以避免重複記錄，如同方位持續鳴叫個體視爲同一個體。有時貓頭鷹受回播引誘而鳴叫聲愈來愈接近調查者，亦視爲同一個體。除非同方位鳴叫聲增加，才記爲另一鳴叫個體。

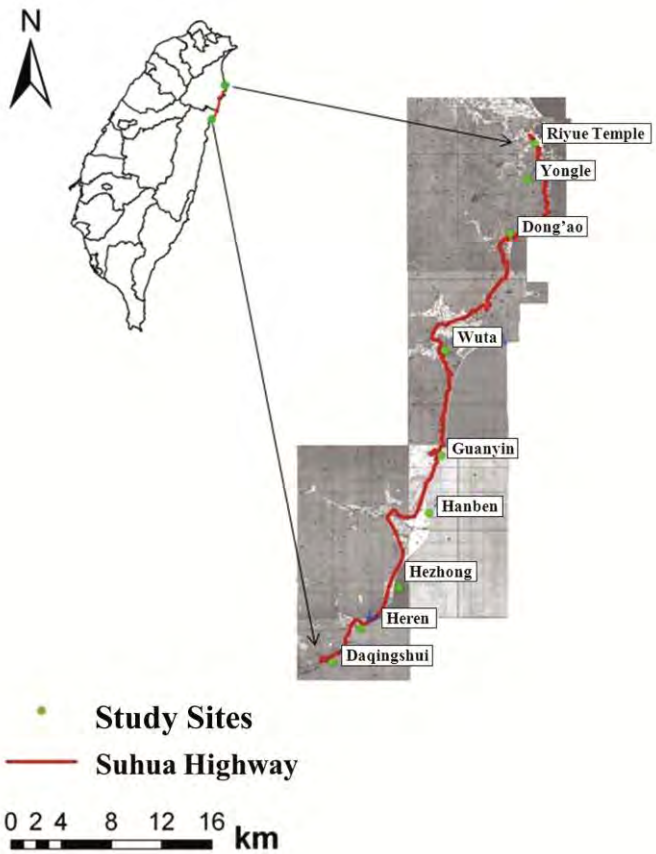

圖 1. 台 9 線蘇澳至大清水段之猛禽各調查樣點位置圖。

Fig. 1. The nine study sites along Suhua Highway.

本研究自 2012 年 3 月起，以 3 個月爲一季的頻度進行調查，3 月至 5 月爲春季，6 月至 8 月爲夏季，9 月至 11 月爲秋季，12 月至隔年 2 月爲冬季，每季調查上午及下午各 1 次，每季共進行 2 次調查。每次調查爲期 6 天，春季 2 次調查分別於 2012 年 3 月 16 日至 21 日及 4 月 23 日至 28 日進行。夏季第 1 次調查於 2012 年 6 月 9 日至 10 日進行，之後因天候不佳，另於 7 月 1 日至 4 日補未完成之調查；第 2 次調查於 8 月 17 至 22 日進行調查。秋季調查分別於 2012 年 9 月 10 日至 15 日及 10 月 8 日至 13 日進行調查。冬季調查分別於 2013 年 1 月 7 日至 12 日及 2 月 1 日至 6 日進行調查。

結 果

本調查2012年3月至2013年2月於台9線蘇花公路蘇澳至大清水段沿線9個調查樣點共記錄到猛禽4科13種497隻次，包括日行性猛禽3科11種447隻次及夜行性猛禽1科2種50隻次(表1)。日行性猛禽中有4種爲留鳥，分別是林鵰(*Ictinaetus malayensis*)、蛇鵰(*Spilornis cheela hoya*)、鳳頭蒼鷹(*Accipiter trivirgatus*)及台灣松雀鷹(*A. virgatus fuscipectus*)；6種爲過境鳥或候鳥，分別是赤腹鷹(*A. soloensis*)、灰面鵟鷹(*Butastur indicus*)、鵟(*Buteo buteo*)、魚鷹(*Pandion haliaetus*)、遊隼(*Falco peregrinus*)及紅隼(*F. tinnunculus*)。另外一種東方蜂鷹(*Pernis ptilohynchus*)，則可能有留鳥或候鳥2種型態。2種夜行性猛禽皆爲留鳥，分別是黃嘴角鴞及領角鴞(附錄2)。所有記錄的日行性猛禽物種中，蛇鵰爲最普遍猛禽，共記錄到228隻次，占所有日行性猛禽總隻次的51%；其次爲鳳頭蒼鷹記錄到78隻次，占所有日行性猛禽總隻次的17%，這兩種猛禽在9個調查樣點中皆可發現(表1)。雖然灰面鵟鷹記錄數量上多於鳳頭蒼鷹，但其僅在過境期出現。夜行性猛禽中，黃嘴角鴞爲最普遍夜行性猛禽，在所有調查樣點中皆可發現，調查期間共記錄到37隻次，占所有夜行性猛禽總隻次的74%(表1)。

春季於 2012 年 3 月至 5 月進行調查，9個調查樣點共記錄日行性猛禽 3 科 7 種 151 隻次及夜行性猛禽 1 科 1 種 4 隻次(表 2)。日行性猛禽以蛇鵰記錄到的數量 106 隻次最多，占春季日行性猛禽全部個體數的 70.2%，其次鳳頭蒼鷹 34 隻次占 22.5%，其他依序爲東方蜂鷹占 2.6%，台灣松雀鷹、魚鷹及遊隼各占 1.3%，最後鵟占 0.7%。夜行性猛禽僅記錄黃嘴角鴞一種。整體而言，春季猛禽結構主要以棲留性猛禽及少數過境猛禽組成，其中以蛇鵰爲優勢物種。

春季 9 個調查樣點中，以大清水樣點之猛禽物種數(圖 2)及個體數(圖 3)最高，蛇鵰爲其中優勢鳥種。觀音樣點亦記錄到 6 種猛禽，但總個體數偏少；另外，和中樣點記錄 4 種，永樂樣點和武塔樣點各記錄到 3 種，蘇澳日月宮樣點、東澳樣點及漢本樣點各記錄到 2 種，和仁樣點僅記錄 1 種。除了觀音樣點和和仁樣點外，其它樣點以蛇鵰最爲優勢。

夏季於 2012 年 6 月至 8 月進行調查，9個調查樣點共記錄日行性猛禽 2 科 6 種 71 隻次及夜行性猛禽 1 科 2 種 17 隻次(表 2)。日行性猛禽以蛇鵰記錄到的數量 46 隻次最多，占夏季日行性猛禽全部個體數的 64.8%，其次鳳頭蒼鷹 19 隻次占 26.8%，其他依序爲東方蜂鷹和台灣松雀鷹各占 2.8%，灰面鵟鷹和遊隼各占 1.4%。夜行性猛禽則記錄到黃嘴角鴞和領角鴞 2 種，其中以黃嘴角鴞記錄各體數最多。整體而言，夏季猛禽結構主要以棲留性猛禽組成，其中以蛇鵰爲優勢物種。

夏季 9 個調查樣點的猛禽物種數以永樂樣點爲最多，記錄到 6 種，以蛇鵰最優勢。其他樣點記錄猛禽物種數介於 2-4 種之間。除了觀音樣點外，其他樣點以蛇鵰最爲優勢。

秋季於2012年9月至11月進行調查，9個調查樣點共記錄日行性猛禽3科9種165隻次及夜行性猛禽1科2種21隻次(表2)。日行性猛禽以灰面鵟鷹記錄到的數量101隻次最多，占秋季日行性猛禽全部個體數的61.2%，其次蛇鵰36隻次占21.8%，其他依序爲鳳頭蒼鷹占8.5%，遊隼占3.6%，台灣松雀鷹占1.8%，魚鷹占1.2%，東方蜂鷹、赤腹鷹及紅隼各占0.6%。夜行性猛禽則記錄到黃嘴角鴞和領角鴞2種，其中以黃嘴角鴞記錄各體數最多。整體而言，秋季猛禽結構主要以過境猛禽和棲留性猛禽組成，其中以過境猛禽灰面鵟鷹爲優勢物種。

表 1. 台 9 線蘇澳至大清水段各樣點之日行性及夜行性猛禽種類及數量

Table 1. Individuals, species and diversity of raptors recorded in the nine study sites

物種 Species	樣點 Sites									總隻次 Total individuals
	日月宮 Riyue Temple	永樂 Yongle	東澳 Dong'ao	武塔 Wuta	觀音 Guanyin	漢本 Hanben	和中 Hezhong	和仁 Heren	大清水 Daqingshui	
林鵰 Indian Black Eagle	-	-	-	-	1	-	-	-	-	1
蛇鵰 Crested Serpent Eagle	9	29	14	52	21	37	28	9	29	228
東方蜂鷹 Oriental Honey Buzzard	-	3	-	-	2	6	-	-	1	12
鳳頭蒼鷹 Crested Goshawk	7	11	2	5	16	4	13	4	16	78
台灣松雀鷹 Besra	-	4	-	-	1	-	-	-	2	7
魚鷹 Osprey	2	-	-	-	-	-	1	-	1	4
灰面鵟鷹 Grey-faced Buzzard	-	94	-	-	7	1	-	-	-	102
赤腹鷹 Chinese Goshawk	-	-	-	-	-	1	-	-	-	1
鵟 Common Buzzard	-	-	-	-	1	-	-	-	-	1
遊隼 Peregrine Falcon	-	1	-	2	2	4	-	-	2	11
紅隼 Common Kestrel	-	-	-	-	-	2	-	-	-	2
領角鴞 Collared Scops Owl	-	6	-	2	-	1	1	1	2	13
黃嘴角鴞 Mountain Scops Owl	-	6	5	8	2	1	6	3	6	37
總物種數 Total species	3	8	3	5	9	9	5	4	8	13
總隻次 Total individuals	18	154	21	69	53	57	49	17	59	497

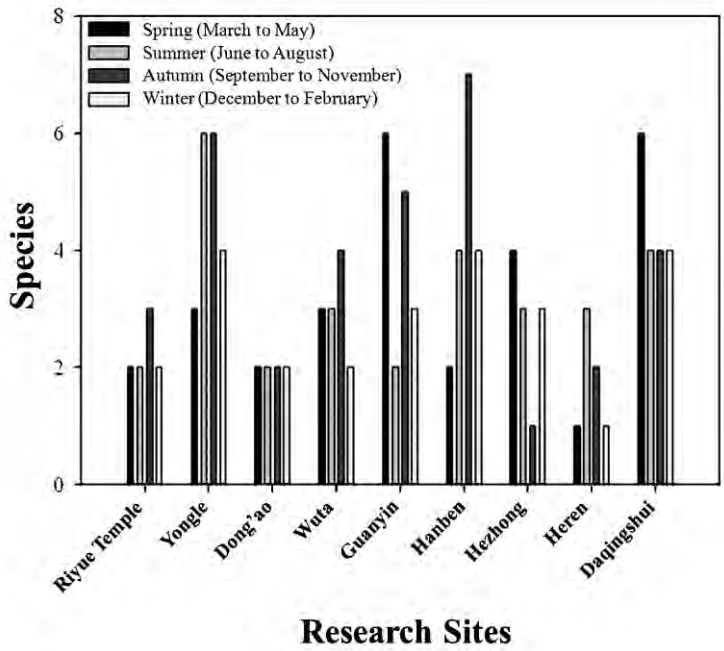

圖 2. 台 9 線蘇澳至大清水段各調查樣點不同季節之猛禽物種數。

Fig. 2. Species of raptors recorded among different seasons in the nine sites.

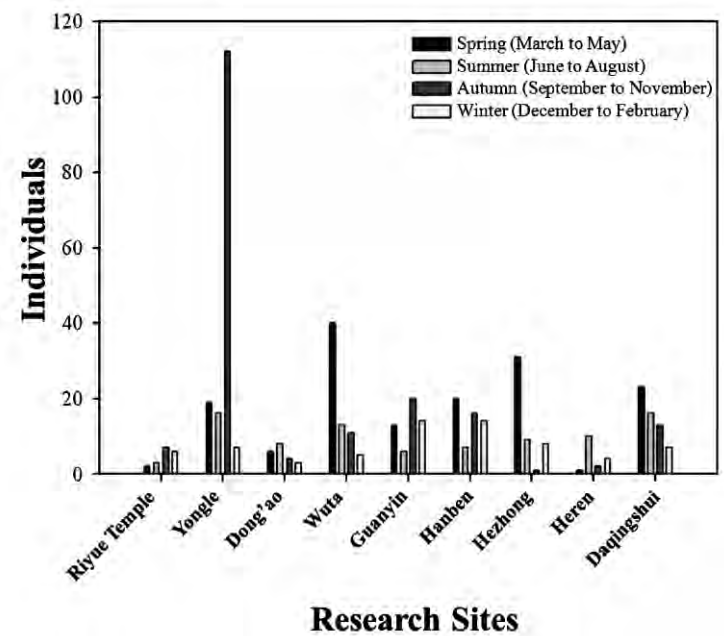

圖 3. 台 9 線蘇澳至大清水段各調查樣點不同季節之猛禽個體數。

Fig 3. The individuals of raptors among different seasons in the nine sites.

表 2. 各季節日行性及夜行性猛禽種類及數量

Table 2. Individuals, species and diversity of raptors recorded during different seasons

物種 Species	季節 Season				總隻次 Total individuals
	春季 Spring	夏季 Summer	秋季 Autumn	冬季 Winter	
林鵰 Indian Black Eagle	-	-	-	1	1
蛇鵰 Crested Serpent Eagle	106	46	36	40	228
東方蜂鷹 Oriental Honey Buzzard	4	2	1	5	12
鳳頭蒼鷹 Crested Goshawk	34	19	14	11	78
台灣松雀鷹 Besra	2	2	3	-	7
魚鷹 Osprey	2	-	2	-	4
灰面鵟鷹 Grey-faced Buzzard	-	1	101	-	102
赤腹鷹 Chinese Goshawk	-	-	1	-	1
鵟 Common Buzzard	1	-	-	-	1
遊隼 Peregrine Falcon	2	1	6	2	11
紅隼 Common Kestrel	-	-	1	1	2
領角鴞 Collared Scops Owl	-	2	6	5	13
黃嘴角鴞 Mountain Scops Owl	4	15	15	3	37
總物種數 Total species	8	8	11	8	13
總隻次 Total individuals	155	88	186	68	497

秋季 9 個調查樣點的猛禽物種數，以漢本樣點記錄到 7 種最多，以蛇鵰最優勢。永樂樣點記錄到 6 種，其中以灰面鵟鷹最優勢，武塔樣點、觀音樣點及大清水樣點各記錄到 4 種，其他樣點記錄鳥種數則在 1-3 種之間。

冬季於 2012 年 12 月至 2013 年 2 月進行調查調查，9 個調查樣點共記錄到 2 科 6 種 60 隻次。日行性猛禽以蛇鵰記錄到的數量 40 隻次最多，占冬季日行性猛禽全部個體數的 66.7%，鳳頭蒼鷹數量次之，占 18.3 %；其他依序爲東方蜂鷹 8.3%，遊隼占 3.3%，林雕及紅隼各占 1.7%。夜行性猛禽則記錄到黃嘴角鴞和領角鴞 2 種，其中以領角鴞記錄個體數最多。整體而言，冬季主要由棲留性猛禽及少數度冬猛禽組成，其中以蛇鵰爲優勢物種。

冬季 9 個調查樣點的猛禽物種數普遍偏低，以永樂樣點、漢本樣點及大清水樣點之猛禽物種數 4 種最高，其它樣點記錄鳥種數則在 1-3 種之間。

全部樣點的每次調查之猛禽物種數(圖 4)及個體數(圖 5)顯示，3 至 4 月中旬猛禽物種數及個體數較多，此時期爲春過境期和繁殖期。之後至 8 月的猛禽物種數及個體數均較少。9 月之後，猛禽物種數及個體數有增加趨勢，其中秋過境期灰面鵟鷹的出現，使得個體數呈現大幅度波動。10 月過後，猛禽物種數及個體數又趨於平緩。台灣過去其他地區之猛禽資源調查結果，如南投九九峰自然保留區(張及林

2005)或嘉義鰲鼓溼地(蔡 2007)，亦呈現春、秋過境期猛禽物種數達到高峰。除了候鳥或過境猛禽物種數及個體數有季節性變化外，記錄數量較多的棲留性猛禽蛇鵰，其觀察個體數因不同季節而有差異，其中春季記錄數量明顯高於其他三季(表 2)。由於春季爲繁殖期前的求偶與配對階段，此時爲蛇鵰一整年最活躍的時期(林 2012)。本研究曾於 2012 年 4 月 27 日於武塔樣點同時觀察到 18 隻個體在空中盤旋。

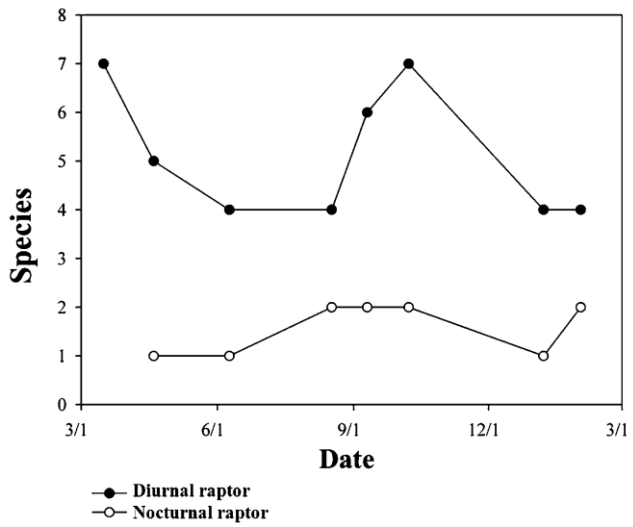

圖 4. 全部樣區的猛禽物種數隨日期之變化。

Fig. 4. The species of raptors varied with the date.

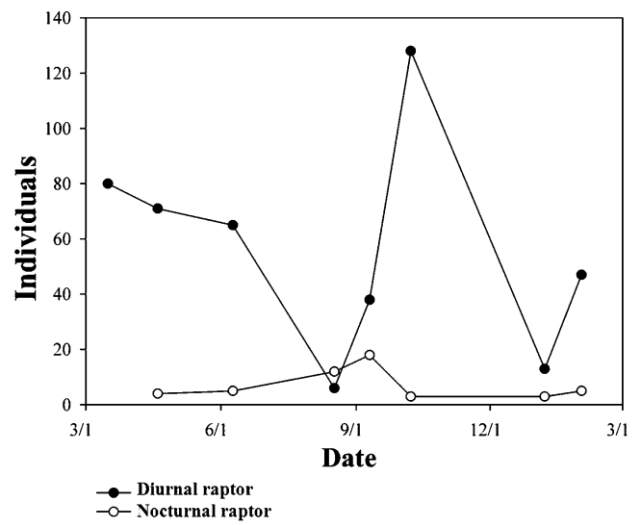

圖 5. 全部樣區的猛禽個體數隨日期之變化。

Fig. 5. The individuals of raptors varied with the date.

在一天之中，日行性猛禽大都於上午時段(8 點～11 點)活動較頻繁，此時段共記錄 9 種猛禽 302 隻次(表 3)。常見日行性猛禽，如蛇鵰(86%)和鳳頭蒼鷹(90%)在上午最爲活躍，下午時段則鮮少活動。日行性猛禽於一天中有 2 個活動高峰期，分別是上午時段的 8 點至 10 點及下午時段的 2 點至 3 點，又以 8 點至 10 點所觀察到的物種數及個體數最多(圖 6)。每種猛禽爲了因應不同的目的，如獵食、求偶、遷移及宣示領域等，都有多樣的飛行方式(Newton 2008)。猛禽的飛行方式大致可分爲六種：鼓翼(flap)、盤旋(soar)、滑翔(glide)、定點(hover)、俯衝(dive)及展示(display)(林 2006)。其中盤旋(70%)是大部分日行性猛禽的主要飛行方式，目前觀察隻次最多的蛇鵰(79%)及鳳頭蒼鷹(69.2%)也都以盤旋爲主要的飛行方式(表 4)。體型愈大的猛禽，如蛇鵰在空中盤旋時間長且體型大，其習性善於鳴叫，較容易被調查人員發現。鳳頭蒼鷹在空中盤旋時間短且體型小，因此較不容易發現。

表 3. 日行性猛禽上午及下午之出現百分比

Table 3. The percentage of diurnal raptors appeared in morning and afternoon

物種 Species	上午 Morning 0800~1100	下午 Afternoon 1400~1700	總隻次 Total individuals
林鵰 Indian Black Eagle	-	1	1
蛇鵰 Crested Serpent Eagle	196(86%)	32(14%)	228
東方蜂鷹 Oriental Honey Buzzard	12	-	12
鳳頭蒼鷹 Crested Goshawk	70(90%)	8(10%)	78
台灣松雀鷹 Besra	6(86%)	1(14%)	7
魚鷹 Osprey	3(75%)	1(25%)	4
灰面鵟鷹 Grey-faced Buzzard	7(7%)	95(93%)	102
赤腹鷹 Chinese Goshawk	1	-	1
鵟 Common Buzzard	1	-	1
遊隼 Peregrine Falcon	6(55%)	5(45%)	11
紅隼 Common Kestrel	-	2	2
總隻次 Total individuals	302(68%)	145(32%)	447

9 個調查樣點以回播法共記錄到 2 種貓頭鷹共 31 隻次，分別爲黃嘴角鴞 20 隻次，領角鴞 11 隻次；黃嘴角鴞在回播前記錄到自發性鳴叫有 17 隻次，領角鴞則有 2 隻次(表 5)。黃嘴角鴞爲蘇花公路蘇澳至大清水段沿線最普遍的夜行性猛禽，除了蘇澳日月宮樣點尚未記錄到以外，其他 8 個調查點皆有記錄。2 種貓頭鷹在調查期間只有鳴叫聲紀錄，並無實際目擊紀錄。黃嘴角鴞回播前所察覺之隻次與回播後察覺之隻次並無顯著差異(Wilcoxon Signed Ranks Test, $Z = 0.597$, $P > 0.05$)；領角鴞則在回播後所察覺之次數明顯高於回播前之記錄(Wilcoxon Signed Ranks Test, $Z = 2.496$, $P < 0.05$)。黃嘴角鴞和領角鴞都對播放同種類的鳴

叫聲有較多回應，其中黃嘴角鴞對同種類的鳴叫聲回應有 9 次，領角鴞則有 5 次，且 2 種貓頭鷹對其他貓頭鷹的鳴叫聲亦會有反應(表 5)。

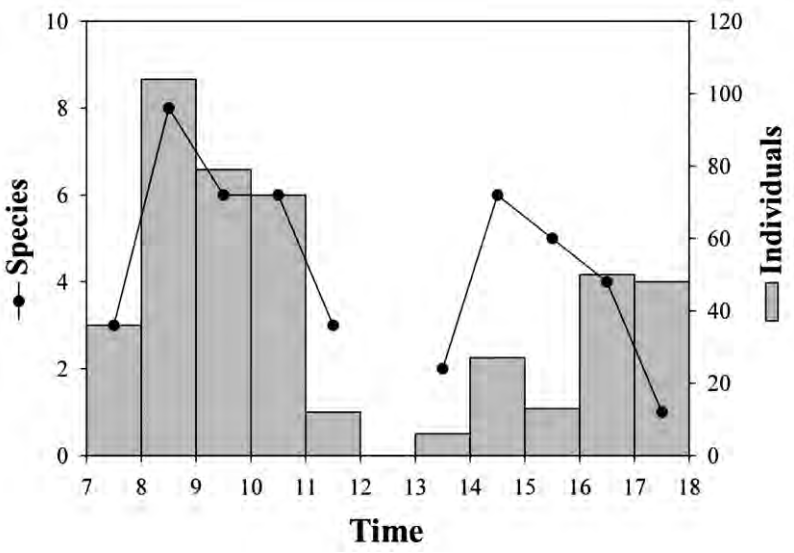

圖 6. 一天中各時段所觀察之日行性猛禽物種數及個體數。

Fig. 6. The species and individuals of diurnal raptors in the time of day.

表 4. 日行性猛禽的行爲模式百分比

Table 4. Percentage of behavior patterns of diurnal raptors

物種 Species	行爲模式 Behavior patterns								總隻次 Total individuals
	鼓翼 Flap	盤旋 Soar	滑翔 Glide	懸停 Hover	俯衝 Dive	展示 Display	停棲 Perch	飛／追擊 Chase	
林鵰 Indian Black Eagle	-	-	1	-	-	-	-	-	1
蛇鵰 Crested Serpent Eagle	25(11%)	180(79%)	14(6.1%)	-	1(0.4%)	-	8(3.5%)	-	228
東方蜂鷹 Oriental Honey Buzzard	7(58.3%)	4(33.3%)	-	-	-	1(8.3%)	-	-	12
鳳頭蒼鷹 Crested Goshawk	6(7.7%)	54(69.2%)	10(12.8%)	-	1(1.3%)	-	6(7.7 %)	1(1.3%)	78

台灣松雀鷹 Besra	-	6(85.7%)	1(14.3%)	-	-	-	-	-	7
魚鷹 Osprey	4	-	-	-	-	-	-	-	4
灰面鵟鷹 Grey-faced Buzzard	38(37.3%)	64(62.7%)	-	-	-	-	-	-	102
赤腹鷹 Chinese Goshawk	1	-	-	-	-	-	-	-	1
鵟 Common Buzzard	-	1	-	-	-	-	-	-	1
遊隼 Peregrine Falcon	4(36.4%)	3(27.3%)	2(18.2%)	-	-	-	-	2(18.2%)	11
紅隼 Common Kestrel	-	1(50%)	-	1(50%)	-	-	-	-	2
總隻次 Total individuals	85(19%)	313(70%)	30(6.7%)	1(0.2%)	2(0.4%)	1(0.2%)	14(3.1%)	3(0.7%)	447

表 5. 2 種夜行性猛禽對回播反應次數

Table 5. Survey of two nocturnal raptors' responses to broadcasting

物種 Species \ Species of playback 回播物種	鵂鶹 Collared Pigmy Owlet	黃嘴角鴞 Mountain Scops Owl	領角鴞 Collared Scops Owl	褐鷹鴞 Brown Hawk Owl	自發性鳴叫 Spontaneous call
黃嘴角鴞 Mountain Scops Owl	6	11	2	1	17
領角鴞 Collared Scops Owl	2	1	5	3	2

討 論

(一) 蘇花公路沿線之猛禽組成

本調查於台 9 線蘇澳至大清水段的日、夜行性猛禽共記錄 4 科 13 種，根據過去文獻資料「烏石鼻海岸自然保護區動物相調查研究」(周 1993)、「和平水泥專用港開發計畫環境影響評估」(財團法人中華顧問工程公司 1994)、「和中電廠發電計畫環境影響說明書」(和中電力股份有限公司 1996)、「太魯閣國家公園蘇花公路沿線動物資源調查暨動物解說文稿」(楊 2002)、「烏石鼻海岸自然保留區動、植物資源

與群聚之研究」(毛及陳 2009)及「臺 9 線蘇花公路山區路段改善計畫生態環境調查報告書」(弘益生態有限公司 2010)，台 9 線蘇澳至大清水段共計有日行性猛禽 3 科 11 種，分別是鶚科的魚鷹；鷹科的林鵰、蛇鵰、東方蜂鷹、鳳頭蒼鷹、台灣松雀鷹、灰面鵟鷹、赤腹鷹及北雀鷹(*Accipiter nisus*)，隼科的遊隼和紅隼；夜行性猛禽 1 科 3 種，分別是鵂鶹、黃嘴角鴞及領角鴞，其中北雀鷹和鵂鶹，爲本研究尚未記錄之物種。北雀鷹爲稀有過境猛禽在春、秋兩季過境期較有機會發現，且出現的隨機性很高。鵂鶹雖爲屬鴟鴞科的夜行性猛禽，但其白天和夜晚皆會活動，且白天夜晚活動比例依季節不同(曾及林 2010)，依目前本調查之研究方法對鵂鶹的察覺度有限。

(二) 猛禽的季節分布

一般而言，春、秋兩季爲猛禽遷徙的季節，冬季則有部分候鳥猛禽度冬，由於過境或度冬猛禽的加入，會使得猛禽物種組成結構的改變。蘇花公路蘇澳至大清水段過去有關遷移性猛禽的調查記錄有限，可能是此路段山勢陡峭道路蜿蜒，適合觀察猛禽的位置有限，亦或非位於遷移性猛禽的主要遷移路徑上，過境猛禽之物種數量較少且零散。在調查期間共記錄到 7 種日行性過境或候鳥猛禽，共 133 隻次，占所有日行性猛禽總隻次的 29.8%，包括春季 4 種，占春季日行性猛禽總隻次的 6%；夏季 3 種，占夏季日行性猛禽總隻次的 5.6%；秋季 6 種，占秋季日行性猛禽總隻次的 67.9%；及冬季 3 種，占冬季日行性猛禽總隻次的 13.3%(表 2)。雖然東方蜂鷹和遊隼爲遷移性猛禽，但此 2 種在研究期間各季節皆有記錄，可能有部分爲留鳥個體，其中東方蜂鷹在台灣已有繁殖生態研究紀錄(黃 2000；劉等 2007)。

在猛禽秋過境期間，根據「和平水泥專用港開發計畫環境影響評估」(財團法人中華顧問工程公司 1994)報告顯示，1990 至 1991 年於和平溪南岸進行鳥類調查期間，曾於 1990 年 9 月及 10 月記錄到赤腹鷹及灰面鵟鷹。本研究於 2012 年 9 月 13 日漢本樣點調查時，觀察到 1 隻赤腹鷹，但整個過境期(9～10 月)也僅在漢本樣點記錄 1 隻。2012 年 10 月 8 日於蘇澳永樂車站樣點進行日行性猛禽調查時，在傍晚 16 時 50 分發現約 94 隻灰面鵟鷹從東北方海面飛入陸地後，在永樂樣點附近山區聚集盤旋，之後降落停棲於樹林。根據灰面鵟鷹遷移研究(*Shiu et al.* 2006, Wu *et al.* 2006)顯示，有些灰面鵟鷹族群會沿著琉球群島進入台灣，部分個體則會在宮古島、石垣島等島嶼度冬。因此推測永樂樣點所觀察到的灰面鵟鷹族群可能是沿著日本西南的琉球群島遷移，此時抵達蘇澳永樂樣點的灰面鵟鷹群因體力耗損而在此停棲休息。停棲於永樂樣點附近山區的灰面鵟鷹是因天候因素或其它因素而臨時在此短暫停棲，還是長期以來的中途停棲點，因過去並無相關調查記錄，所以必須長期於過境期調查才能確定。

本調查僅在蘇澳永樂車站觀察到數量較大的灰面鵟鷹群，而漢本樣點只記錄到灰面鵟鷹零星個體及 1 隻赤腹鷹，亦有可能灰面鵟鷹及赤腹鷹由東北部進入台灣後並非沿著較靠近海岸的蘇花公路沿線的山脈遷徙，而是沿著較內陸的山脈遷徙，但這必須經長期調查才能確認。由於本研究於春、秋過境期間並無針對過境猛禽進行密集完整的調查，故無法反映春、秋兩季實際猛禽過境的狀況，亦有可能是赤腹鷹和灰面鵟鷹春、秋兩季的遷移路徑不同所致。未來在春過境期或秋過境期，需藉由適當增加努力量，才能反映過境期猛禽群聚結

構。

(三) 各調查樣點猛禽分布

根據洪等(2010)針對大型猛禽棲地之預測顯示，林鵰和東方蜂鷹偏好利用較陡的山區原始林，其中東方蜂鷹亦適應較破碎化的森林。蛇鵰雖利用山區森林爲主，但亦適應更多破碎化或干擾之森林。鳳頭蒼鷹利用之棲地則從原始林至都市綠地(黃等 2008；胡 2009)。由於蛇鵰和鳳頭蒼鷹對棲地的耐受度較高，因此這2種猛禽在9個調查樣點中皆可發現。本研究觀察猛禽物種數最多的觀音樣點及漢本樣點，棲地類型屬較陡的山區原始林，其中觀音樣點曾記錄林鵰，該地區因地勢較陡峭，人爲開發較少，在植被上保留了較豐富的天然闊葉林環境(弘益生態有限公司 2010)。觀音及漢本樣點因山勢陡峭人爲干擾少，保留較豐富的天然闊葉林，蘇澳日月宮樣點於蘇澳市區內人爲開發及干擾較大，所觀察到的猛禽爲對環境耐受度較高之蛇鵰和鳳頭蒼鷹。

蘇花公路沿線9個樣點間的猛禽種數及族群數量差異大，且各樣點分布不平均，由於本研究僅進行第1年調查，且過去並無調查報告參考，故尚無法完整了解蘇花公路沿線之猛禽族群趨勢。未來待持續定期調查後，以期更能瞭解猛群族群趨勢是否隨環境變遷而改變。

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附錄 1. 台 9 線蘇花公路蘇澳－大清水段各調查樣點之座標

Appendix 1 The coordinates of nine samples along Suhua Highway

樣點	TWD97	
	X	Y
大清水樣點	320000	2679456
和仁樣點	322307	2682090
和中樣點	325355	2685419
漢本樣點	327685	2691320
觀音樣點	328641	2695930
武塔樣點	328956	2704348
東澳樣點	334154	2713671
永樂樣點	335504	2717994
蘇澳日月宮樣點	336125	2720853

附錄 2. 台 9 線蘇花公路蘇澳至大清水段各調查樣點記錄之猛禽物種

Appendix 2. The species of raptors recorded from the nine sites along Suhua Highway

中文名	學名	保育[1]	生息狀態[2]		調查樣點								
		等級	留鳥	候鳥	大清水	和仁	和中	漢本	觀音	武塔	東澳	永樂	日月宮
鷹科	Accipitridae												
林鵰	*Ictinaetus malayensis*	I	R						●				
蛇鵰	*Spilornis cheela hoya*	II	C		●	●	●	●	●	●	●	●	●
東方蜂鷹	*Penis ptilohynchus*	II	U	C	●			●	●			●	
鵟	*Buteo buteo*	II		U					●				
鳳頭蒼鷹	*Accipiter trivirgatus*	II	C		●	●	●	●	●	●	●	●	●
台灣松雀鷹	*Accipiter virgatus fuscipectus*	II	U		●				●			●	
赤腹鷹	*Accipiter soloensis*	II		C				●					
灰面鵟鷹	*Butastur indicus*	II		C				●	●			●	
鶚科	Pandionidae												
魚鷹	*Pandion haliaetus*	II		U	●		●						●
隼科	Falconidae												
遊隼	*Falco peregrinus*	I		U	●			●	●	●		●	
紅隼	*Falco tinnunculus*	II		C				●					
鴟鴞科	Strigidae												
領角鴞	*Otus lettia*	II	C		●	●	●	●		●		●	
黃嘴角鴞	*Otus spilocephalus*	II	C		●	●	●	●	●	●	●	●	

物種數	4 科 13 種	8	4	4	9	9	5	3	8	3

[1] 依據行政院農業委員會於民國 98 年公告之保育類野生動物名錄，保育等級分爲：I 表示瀕臨絕種野生動物；II 表示珍貴稀有野生動物；III 表示其他應予保育之野生動物。Protected Species are classified into three categories: I: Endangered Species; II: Rare and Valuable Species; III: Other Conservation-Deserving Wildlife.

[2] 生息狀態 Status：C –普遍(Common)；U –不普遍(Uncommon)；R –稀有(Rare) (林 2006；曾及林 2010；廖 2012)。

Trends of Freshwater Crabs and Habitats in Southern Taiwan after Typhoon Morakot

莫拉克颱風過後臺灣災區淡水蟹類及棲地之時序變化趨勢

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Abstract

Natural disasters can cause severe influences on the ecosystem through different spatial and temporal scales. Decades of forest fire researches have been conducted to understand the effects of natural disaster on the ecosystem. However, the effects of typhoon upon freshwater ecosystem remain unclear, and the difficulty is exacerbated by the impacts of global warming and climate change. Furthermore, freshwater crabs are perhaps in a much difficult situation because their most important biodiversity occurs in locations where the most destructive typhoons happen, but there is still no research focusing on it. Typhoon Morakot passed through Taiwan in August 2009, and the heavy rainfall brought by the typhoon resulted in serious floods, debris flows and landslides in southern Taiwan. From spring 2010 to summer 2013, we investigated the trends of freshwater crabs and their habitats after Typhoon Morakot using systematic sampling and analyzing methods for six individual and community indicators (body size, sex,

maturity, community biodiversity, community abundance and community biomass). Our results revealed highly increasing community abundance and marginally increasing community biomass in freshwater crabs, which indicated that the numbers of freshwater crabs and their habitat resources were recovering after typhoon. Interestingly, the constraints of gradually ascending community biomass and drastically rising community abundance resulted in the“side-effect” of body size decline in freshwater crabs. To reduce extinction risks in a dramatically fluctuating environment, we surmise that freshwater crabs shift their adaptive strategy toward r-selected life history to benefit their reproductive success in face of extreme catastrophes like Typhoon Morakot.

摘　要

經由數十年森林火災相關研究瞭解，偶發性大天災常造成生態系於不同時空尺度的重大影響。但於現今全球暖化與氣候變遷影響加速下，卻少有研究探討漸趨惡化的颱風天災與淡水域生態系間相關效應，而特別針對最可能遭受颱風侵襲而喪失重要生物多樣性資源的淡水蟹類，至今仍無相關研究。臺灣於 2009 年 8 月遭遇莫拉克颱風襲擊，造成臺灣歷史上最嚴重的水患、土石流及山崩災情。本研究即於莫拉克颱風過後，運用系統性取樣 22 處災區研究樣站，除於 2010 年 1 月風災後初期調查淡水蟹類的基線資料外，並自 2011 年春季至 2013 年夏季期間每季持續進行調查淡水蟹類個體及群聚的 6 項相關指標（體型、性別、成熟、群聚生物多樣性、群聚豐富度及群聚生物量），用以分析其於風災後的時序變化趨勢。研究結果發現淡水蟹類群聚豐富度顯著上升與群聚生物量近顯著增加的趨勢，顯示風災後的淡水蟹類數量與其棲地資源正在恢復，另經由時間序列分析發現，受限於緩慢增加的群聚生物量與快速上升的群聚豐富度，造成淡水蟹類體型小型化的附帶效應。我們推測在極具破壞性的天災發生後，如同莫拉克颱風所造成的巨大災難，淡水蟹類可快速增加個體數量，主動調整適應策略朝向 r 型選汰生活史模式，藉以提高其子代於更趨劇烈變化環境中的存活機率。

Key words: freshwater crab, freshwater ecosystem, Typhoon Morakot, recovery

關鍵詞：淡水蟹類、淡水域生態系、莫拉克颱風、復原

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Introduction

Natural disasters can affect ecosystems remarkably. Forest fire is one of the best studied topics in natural disasters. Fire regime is realized not only to play a crucial role in the forest ecosystem structure (Ahlgren and Ahlgren 1960; Agee 1996), but also to influence the forest ecosystem function via chemical and physical dynamics of the environment (Johnson and Miyanishi 2001). In addition, fire enhances transformation of forest ecosystem structure and function through space as well as time (Whelan 1995). Tropical cyclone, known as hurricane or typhoon by its originating location, is another important cause of nature disasters. Tropical cyclone effects on forest ecosystem are also well documented in the literature. It can acutely and dramatically affect forest populations (Horvitz *et al.* 1995), communities (Pascarella 1998) and ecosystems (Loope *et al.* 1994). For example, defoliation is the most common effect of tropical cyclone on forest structure, and typhoon strength accounts for 82% of the litter fall variation in Fushan Experimental Forest in Taiwan (Lin *et al.* 2003).

Tropical cyclone effects on distinct ecosystems such as forest and freshwater ecosystem, however, are not interchangeably applicable without supporting evidence. The relationship between tropical cyclone and freshwater ecosystem is still unclear, and this is made all the more difficult to understand due to the impacts of global warming and climate change. Ongoing global warming (IPCC 2007) can accelerate the happenings of more intense and frequent tropical cyclone (Emanuel 2005; Webster *et al.* 2005; Elsner *et al.* 2008), and the phenomenon will amplify the ecological impacts on freshwater ecosystem. Along with this impact, freshwater ecosystem is particularly vulnerable to climate change because of its isolation and fragmentation within a largely terrestrial landscape (Woodward *et al.* 2010). Thus, further research is needed to understand tropical cyclone effects on freshwater ecosystem to reduce the double threats.

Furthermore, freshwater crabs of the freshwater ecosystem are highly probable in a much difficult situation because their most important biodiversity occurs in locations where the most destructive tropical cyclones happen, but there is no research focusing on it. Asia, including Taiwan is the biodiversity global center and hotspot of freshwater crabs (Cumberlidge *et al.* 2009). As such, it has not only the most abundant but also the most endangered freshwater crab species in this area. At the same time, the highest number of tropical cyclones in the world are formed in western tropical Pacific Ocean (Chan *et al.* 2004), and their paths follow three general directions that majorly affect Southeast and East Asia (Elsner and Liu 2003) where the most important biodiversity of freshwater crabs occurs. Therefore, it becomes urgent to study the responses of freshwater crabs to tropical cyclone in order to determine if these catastrophic events would threaten the survival of freshwater crabs.

Typhoon Morakot, equivalent to category 1

on the Saffir-Simpson Hurricane Scale (Simpson and Riehl 1981), passed through Taiwan in August 2009, and it was the most devastating typhoon in Taiwan's historical records due to the tremendous amount of rainfall it brought from August 6 to 11. The extremely heavy rainfall induced lots of serious floods, debris flows and landslides in southern Taiwan in places where many unique freshwater crabs exist. Hence Typhoon Morakot might have rapidly increased the risk of extinction of freshwater crabs in Taiwan.

This study is the first research discussing the effects of typhoon on freshwater crabs. Using systematic sampling and analyzing methods for six individual and community indicators (body size, sex, maturity, community biodiversity, community abundance and community biomass) of freshwater crabs from spring 2010 to summer 2013, this study aimed to investigate the recovery trends of freshwater crabs and their habitats after Typhoon Morakot.

Materials and Methods

Study sites

Typhoon Morakot induced floods and debris flows in most major water systems in southern Taiwan. Our study sites were all selected in different affected water systems where crabs could be found alive or defunct. These sites were divided into three types according to different sampling methods (Fig. 1).

I. Historical Study Sites

Sites where crab surveys had been conducted in previous studies. First, Haiqian NO.2 Bridge, located in estuary of Zhongni Stream, Hengchun, Pingtung. Second, Maolin Suspension Bridge, located in northern upstream tributary of Gaoping River, Maolin, Kaohsiung. Third, Jixiang Bridge, located in upstream Gaoping River, Namaxia, Kaohsiung.

II. Monitoring study sites

Sites were selected by their unique freshwater crabs fauna and used to evaluate the species effect on crab indicators. First, South-Link Highway 458K, located in upstream Fenggang River, Shihzih, Pingtung. Second, Laiyi Village, located in upstream Linbian River, Laiyi, Pingtung. Third, Taiwu Village, located in upstream Donggang River, Taiwu, Pingtung. Fourth, Yila Waterfall, Yila Community and Wutai NO.2 Bridge, located in southern upstream tributary of Gaoping River, Wutai, Pingtung.

III. Other study sites

Sites were selected by their habitat difference and used to evaluate the habitat effect on crab indicators. First, CSIST Dormitory, located in downstream Xuhai River near estuary, Mudan, Pingtung. Second, Chunri Village, located in downstream Beishi River, Chunrih, Pingtung. Third, Liangshan Waterfall, located in upstream Donggang River, Majia, Pingtung. Fourth, Sandimen Bridge, located in southern upstream tributary of Gaoping River, Neipu, Pingtung. Fifth, Majia NO.3 Bridge, Majia NO.5 Bridge and Majia Waterfall, located in southern upstream tributary of Gaoping River, Majia,

Pingtung. Sixth, Wutai Watersource, located in southern upstream tributary of Gaoping River, Wutai, Pingtung. Seventh, Guanshan NO.1 Bridge and NO.6 Bridge, located in upstream Gaoping River, Jiasian, Kaohsiung. Eighth, NO.15 Bridge, Minzu Bridge and Nacilan Bridge Upstream 1k, located in upstream Gaoping River, Namaxia, Kaohsiung.

Fig. 1. Geographic distributions of the study sites in this study. Study sites were distributed in different affected water systems in southern Taiwan after Typhoon Morakot and labeled as Historical, Monitoring and Other ones.

Study methods

We conducted surveys in study sites from spring 2010 to summer 2013 to collect freshwater crab data of six individual and community indicators. In each survey, we made exhaustive search but with least harm to collect crabs in 50 meters along riparian upstream from the fixed point of study sites and set six traps overnight. After identifying species and measuring the traits of carapace breadth, carapace length, wet weight, sex and maturity, the captured crabs were released to their native habitats, but ones with doubt in species identification were needed for confirmation in the laboratory. The data were transformed into individual Indicators (body size, sex and

maturity) for each crab data and community Indicators (biodiversity, abundance and biomass) for each study site data. Habitat intactness and water quality of the study sites were also recorded for further study.

It is necessary to mention that community biomass of freshwater crabs, one of the community indicators, can indicate the status of the habitat resource. It can be illustrated by the hypothesis of habitat resource supply and consumption. Total resource consumption by all communities in the habitat is proportional to total resource supply of the habitat, and specific community accounts for particular ratio of total resource consumption. In addition, specific community biomass is proportional to its resource consumption because there is an allometric relationship between individual biomass and its resource consumption (Ernest and Brown 2001; Ernest *et al.* 2003, detailed discussion in the Results). Freshwater crabs, moreover, also comprise a large proportion of the overall biomass of all invertebrates in the tropical streams (Abdallah *et al.* 2004). It was supposed that these relationships would not change after Typhoon Morakot. Thus, the community biomass of freshwater crabs was taken as an indicator of the habitat resource for evaluating its recovery trend.

Data analysis

Individual indicators (body size, sex and maturity) and community indicators (biodiversity, abundance and biomass) of freshwater crabs were mainly analyzed in multiple linear and logistic regression models by JMP 9.0. Using six crab indicators as dependent variables (Y) and time series as independent variable (X), we could evaluate the time effect on crab indicators, and thus obtained the recovery trends of crab indicators after Typhoon Morakot. These factors are listed in Table 1.

Besides major independent variable (X) of time series, we also considered the influences of minor independent factors on crab indicators. These factors included seasons, species, habitat types, time series interaction with habitat types, reciprocal interactions among individual indicators and autoregression of community indicators, which are not further discussed in this study. By setting these minor factors as covariates in the multiple linear and logistic regression models to control their influences, we could confirm the true time effect on the six crab indicators.

If the potential time relationships among crab indicators (Y) exist, time series analysis of panel data is suggested for analyzing these reciprocal relationships. Panel Data Analysis (PDA) or time-series-cross-section (TSCS) data analysis is one of the most popular analyses in econometric research, and it has become popular in other research fields. The advantages principally lie in capacity for modeling cross-sectional and longitudinal (time series) data (Hsiao 2007). Our data of freshwater crab community indicators were fit for panel data analysis, and thus we attempted to progress the analysis in EView 7.2 for better understanding of the temporal relationship among crab indicators.

Table 1. The factors analyzed in multiple linear and logistic regression models in this study

Variables				Explanations
Y / X		Types	Name	
Dependent	**Individual Indicators**	Body Size traits	**Carapace Breadth (CB)**	The widest distance from the opposite ends of anterolateral margins of a crab's carapace, measured in minimeter.
			Carapce Length (CL)	The longest distance from the frontal margin to posterior of a crab's carapace, measured in minimeter.
			Weight (W)	The wet weight of a crab, measured in gram.
			Sex	Differentiate the male and female from the shape of the abdomen and/or gonopod.
			Maturity	Differentiate the adult and juvenile from the body size comparison with smallest hatching adult female of the same crab species.
	Community Indicators	Biodiversity	**Shannon H'**	Habitat's biodiversity was measured through Shannon diversity index (H') in each habitat survey.
		Abundance	**Crab Individuals number (N)**	Habitat's abundance was measured through the counts of crab individuals in each habitat survey.
		Biomass	**Total Crab Weight (TW)**	Habitat's biomass was measured through the sum of total crab weights in each habitat survey.
Independent	**Trend**		**Time Series (TS)**	Time Series was sequentially coded from the first quarter after Typhoon Morakot. Surveys were taken quarterly to examine the trend.
Covariates[a]				The influences of seasons, species, habitat types, time series × habitat types, reciprocal interactions among individual indicators and autoregression of community indicators were controlled as covariates.

Note : a The effects of covariates were controlled in the multiple linear and logistic regression models, but they would not be further discussed in this study.

Results

Summaries of survey frequencies and crab data in each study site are listed in Table 2, and crab faunas are listed in Table 3.

Descriptive statistics of six indicators of freshwater crabs after Typhoon Morakot

The means ± SE of individual and community indicators of freshwater crabs were listed as follows: body size (CB: 18.356 ± 0.155 mm, n=2,797; CL: 15.365 ± 0.130 mm, n=2,797; W: 4.033 ± 0.103 g, n=2,792), sex (male: 55.3 %, female: 44.7 %, n=2,758), maturity (juvenile: 59.6 %, adult: 40.4 %, n=2,539), community biodiversity (Shannon H_e': 0.505 ± 0.036 , n=153), community abundance (N: 15.915 ± 1.234 , n=177) and community biomass (TW: 63.703 ± 6.363 g, n=177).

Time effects on six indicators of freshwater crabs after Typhoon Morakot (Table 4)

I. Individual indicators

Firstly, three body size traits of freshwater crabs, carapace breadth, carapace length and weight, were highly correlated (CB and CL: r_s=0.990, p<0.0001; CB and W: r_s=0.989, p<0.0001; CL and W: r_s=0.987, p<0.0001). It would be adequate for applying principal component analysis to acquire the single indicator representing three body size traits. Principal component 1 (PC1) accounted for the most variance (λ=2.882, variance %=96.078) and had the same pattern with three traits in multiple linear regressions. We used PC1 as the body size indicator in the analysis and found that body size was highly negatively influenced by the time series. Secondly, the time series had no effect on the likelihoods of sex and maturity of freshwater crabs.

II. Community Indicators

Firstly, the time series had no effect on the biodiversity (Shannon H') of freshwater crab communities. Secondly, community abundance of freshwater crabs (N) was highly positively influenced and community biomass of freshwater crabs (TW) was marginally positively influenced by the time series.

Table 2. Survey frequencies and crab data among study sites in this study

Typhoon Morakot		Post - Typhoon													
Time	Year	2010				2011				2012				2013	
	Season	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
	Series	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Study sites															
Haiqian NO.2 Bridge	[a]									6	5	9	10	4	15
CSIST Dormitory	[c]						4		19	16	14	19	23	17	22
South-Link Highway 458K	[b]						15	17	24	20	30	34	55	55	45
Chunri Village	[c]					6			8	0	5	3	3	0	4
Laiyi Village	[b]	4				3	19	8	28	13	26	12	36	11	20
Taiwu Village	[b]	4				4	18	24	61	36	64	44	67	47	84
Liangshan Waterfall	[c]					3			36	29	20	18	15	29	26
Sandimen Bridge	[c]					11			23	11	13	27	16	12	19
Majia NO.3 Bridge	[c]					8			11	3	2	7	1	4	8
Majia NO.5 Bridge	[c]					1			9	3	10	16	14	21	9
Majia Waterfall	[c]					4			16	41	15	28	27	57	22
Yila Waterfall	[b]	8				11	7	12	19	12	8	13	30	54	15
Yila Community	[b]	3				4	9		22	17	13	6	0		2
Wutai NO.2 Bridge	[b]	12				3		4	5	3		8	8	13	11
Wutai Watersource	[c]						1		7	5	4	14	4	1	11
Maolin Suspension Bridge	[a]									0	0	0	0	0	0
Guanshan NO.1 Bridge	[c]					8			15	20	12	19	15	5	13
NO.6 Bridge	[c]					15			12	36	7	21	20	44	17
Jixiang Bridge	[a]									0		0	0	0	0
NO.15 Bridge	[c]								0	0		0	0	0	0
Minzu Bridge	[c]								0	17		0	1	2	1
Nacilan Bridge Upstream 1k	[c]					26			11	22		7	7	20	

Notes : Blank rectangles denote no surveys. Rectangles with slash line denote unconducted surveys because these study sites were not accessible. Rectangles with gray background represent conducted surveys.

Number in rectangles denotes the crab data acquired in each survey.

[a] Historical study sites. [b] Monitoring study sites. [c] Other study sites.

Table 3. Data of freshwater crab species and individual numbers among study sites analyzed in this study

Study sites		Freshwater Crabs										Total
		C. rathbunae	*G. albogilva*	*G. caesia*	*G. ferruginea*	*G. lili*	*G. neipu*	*G. olea*	*G. shernshan*	*G. tawu*	*G. tsayae*	
Haiqian NO.2 Bridge	[a]	21	7	0	21	0	0	0	0	0	0	49
CSIST Dormitory	[c]	42	67	0	25	0	0	0	0	0	0	134
South-Link Highway 458K	[b]	223	0	0	0	0	0	0	0	72	0	295
Chunri Village	[c]	5	2	0	0	2	5	15	0	0	0	29
Laiyi Village	[b]	36	0	0	0	0	68	76	0	0	0	180
Taiwu Village	[b]	108	0	0	0	29	179	137	0	0	0	453
Liangshan Waterfall	[c]	18	0	0	0	2	93	63	0	0	0	176
Sandimen Bridge	[c]	60	0	0	0	47	5	20	0	0	0	132
Majia NO.3 Bridge	[c]	34	0	0	0	1	3	6	0	0	0	44
Majia NO.5 Bridge	[c]	57	0	0	0	26	0	0	0	0	0	83
Majia Waterfall	[c]	209	0	0	0	1	0	0	0	0	0	210
Yila Waterfall	[b]	164	0	0	0	0	0	0	25	0	0	189
Yila Community	[b]	0	0	0	0	0	0	0	76	0	0	76
Wutai NO.2 Bridge	[b]	67	0	0	0	0	0	0	0	0	0	67
Wutai Watersource	[c]	44	0	0	0	2	0	0	1	0	0	47
Maolin Suspension Bridge	[a]	0	0	0	0	0	0	0	0	0	0	0
Guanshan NO.1 Bridge	[c]	29	0	1	0	0	0	1	0	0	76	107
NO.6 Bridge	[c]	94	0	1	0	0	0	0	0	0	77	172
Jixiang Bridge	[a]	0	0	0	0	0	0	0	0	0	0	0
NO.15 Bridge	[c]	0	0	0	0	0	0	0	0	0	0	0
Minzu Bridge	[c]	2	0	0	0	0	0	0	0	0	19	21
Nacilan Bridge Upstream 1k	[c]	93	0	0	0	0	0	0	0	0	0	93
Total		1306	76	2	46	110	353	318	102	72	172	2557

Notes：[a] Historical study sites. [b] Monitoring study sites. [c] Other study sites.

Table 4. The effects of time on six crab individual and community indicators were analyzed in multiple linear and logistic regression models. Body size, community abundance and community biomass of freshwater crabs exhibited more dominant trends than other indicators after Typhoon Morakot

	Individual indicators				Community indicator		
X variables	**Body size** (Principal component 1, PC1) n=2509	**Sex** (log odds (Female / Male))[a] n=2509	**Maturity** (log odds (Juvenile/Adult))[a] n=2509	**Biodiversity** (Shannon H') n=110	**Abundance** (Total individual number, N) n=130		**Biomass** (Total crab weight, TW) n=130
Full model							
R^2adj	0.578	0.049	0.945	0.810	0.702		0.648
df	37	37	37	26	29		29
Time Series							
b ± SE	**-0.164 ±0.020**	0.036 ±0.040	-0.257 ±0.566	0.008 ±0.021	**3.876 ±0.938**		**9.242 ±5.043**
F Ratio / LR_X^2	**68.670**	0.835	0.198	0.123	**17.067**		**3.359**
*p*value	**<0.0001** ***	0.361	0.656	0.727	**<0.0001**	***	**0.070**

Notes: [a] General Equation of Multiple Logistic Regression： $G = \log it(p_{event}) = \log(odds_{event}) = \log(\frac{p_{event}}{1-p_{event}}) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + ... + \beta_i X_i + \varepsilon$

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Panel data analysis of body size, community abundance and community biomass of freshwater crabs after Typhoon Morakot

The effects of time on freshwater crabs were dominant in trends of the three indicators, body size decline, community abundance and community biomass rise, from results of previous section. The relationships among body size, abundance and biomass can be illustrated by the equation of habitat resource supply and resource consumption by populations or communities (Ernest and Brown 2001; Ernest *et al.* 2003):

$$R \cong C_{total} = \sum_{i=1}^{n} C_i \qquad (1)$$

where R is the resource supply of the habitat, C_{total} is the resource consumption by all communities and C_i represents resource consumption by specific community. If we focus on crab community of the habitat, the resource consumed by crabs in a habitat can be expressed as:

$$C_i \cong aB_i = aN_i\overline{M}_i^b \qquad (2)$$

where B_i is the biomass of the crab community, N_i is the number of total individuals of the crab community (abundance), $\overline{M}_i$ is the mean body mass of the crab community (body size: mean weight), a is an allometric constant and b is an allometric exponent. If the typhoon effect will not alter the relationships in the equation 1 and 2, we believe that the community biomass of freshwater crabs can infer their habitat resource, and thus the recovery trend of habitat resource can be assessed by time pattern of crab community biomass.

In order to identify whether the trends of the three indicators resulted from reciprocal influences in equation 2, we further analyzed our data by Vector AutoRegression (VAR) Granger Causality Test of panel data analysis. This test can determine whether one time series data is useful in forecasting the others. Our results revealed that both biomass and abundance would granger cause body size of freshwater crab communities (Table 5).

Table 5. Vector AutoRegression (VAR) Granger Causality among body size, abundance and biomass of freshwater crab communities. Biomass and abundance would granger cause body size of freshwater crab communities

Indicators	**Body size (Mean weight, W)** n=24			**Abundance (Crab individuals number, N)** n=24			**Biomass (Total crab weight, TW)** n=24		
	df	χ^2	*p* value	*df*	χ^2	*p* value	*df*	χ^2	*p* value
Body size				6	2.045	0.915	6	1.889	0.930
Abundance	6	**12.791**	**0.046 ***				6	1.464	0.962
Biomass	6	**17.762**	**0.007 ****	6	0.907	0.989			

Notes: * $p<0.05$, ** $p<0.01$

Moreover, the VAR equation of crab body size could be expressed as:

W = -0.002×N(-1) +0.083×N(-2) -0.154×N(-3)* -0.153×N(-4) -0.091×N(-5) -0.099×N(-6) +0.005×TW(-1) +0.006×TW(-2) +0.022×TW(-3)* +0.005×TW(-4) -0.003×TW(-5) +0.026×TW(-6) +0.387×W(-1) -0.233×W(-2) -0.556×W(-3) +0.288×W(-4) -0.509×W(-5) -0.045×W(-6) +8.778

Five over six lag phases of abundances negatively affected body size, especially for most significant effect of lag 3 (asterisk labeled, χ_1^2= 4.434, p= 0.035). On the contrary, five over six lag phases of biomass positively affected body size, especially for most significant effect of lag 3 (asterisk labeled, χ_1^2= 4.943, p= 0.026).

In equation 2, our results supported the positive effect of biomass and negative effect of abundance on body size. According to the relative stable trend of biomass in result of Table 4, the former's drastically increasing abundance caused the latter rapidly decreasing body size (Fig. 2).

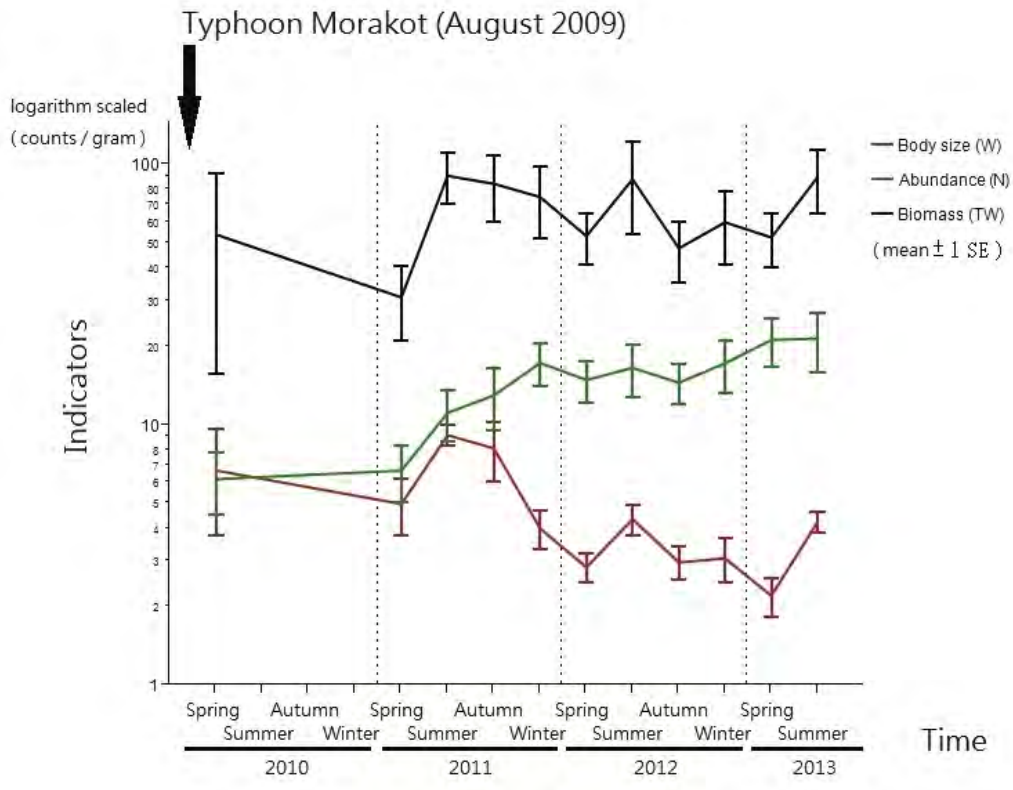

Fig. 2. Drastically rising community abundance (Crab individuals number, N) and gradually ascending community biomass (Total crab weight, TW) resulted in rapidly declining body size (mean weight, W) in freshwater crab communities.

The trend patterns of community abundance rise and body size decline of freshwater crabs were further checked by adding a square of time series as independent variable in their multiple linear regressions. The results revealed that these trends would not change through time (community abundance: $F_{1,99}$=0.323, p=0.571; body size: $F_{1,2471}$=1.598, p=0.206).

Discussion

This study is the first research focusing on the responses of freshwater crabs after typhoon, and we found drastically increasing community abundance and gradually increasing community biomass in freshwater crabs after Typhoon Morakot. It indicated that the numbers of freshwater crabs and their habitat resources were recovering in short-term study period after typhoon, but the recovery in freshwater crabs was much faster than the natural recovery of habitat resource. Despite lack of pre-typhoon information for comparison, the results are consistent with the findings of McMullen and Lytle (2012). They concluded that the abundance of freshwater invertebrate reduces at least one half after flood events, and the negative effect becomes greater with increasing relative flood magnitude. Freshwater invertebrates increase in numbers in short-term period after a flood event, but these patterns vary among different taxonomic groups. They propose the variation patterns of abundance recovery arising from different life history and lifespan of taxonomic groups.

The abundance increase was a result of the higher offspring surviving rate, which involves the adaptive strategies of species. In comparisons with marine crabs, freshwater crabs will undergo direct development in which the large, yolky eggs hatch directly into juvenile crabs and extend their parental cares (Yeo *et al.* 2008). Abbreviation of larval development and extension of parent care are key features of freshwater decapoda such as freshwater crabs, which tends to be k-selected life history with parental investments for best offspring surviving in particularly varied freshwater habitats (Vogt 2013).

Our results, however, revealed an average of 97.4% (3.876 * 4 / 15.915 , Table 4 and first part of the results) increase annually in abundance of freshwater crabs after Typhoon Morakot. We suggest adaptive strategy modification toward r selected life history in freshwater crabs to rapidly recover from the catastrophic damages and reduce the extinction risk in future extreme catastrophes. Typhoon Morakot, the most devastating typhoon in Taiwan's history, induced the most serious floods in affected water systems and caused the highest reduction of abundance in freshwater crabs. If more intense and frequent typhoons or other nature disasters happen due to the impacts of global warming and climate change, the occurrence of extreme catastrophes will be in a much higher condition. Under the usual extinction pressures caused by extreme catastrophes, it will be evolutionarily advantageous to shift to r-selected life history that maximizes reproductive potential in dramatically fluctuating environments.

Interestingly, our results also showed the trend pattern of body size decline in freshwater crabs, which resulted from the constraints of gradual ascending habitat resource and drastically rising abundance. The negative relationship between body size and abundance has been widely recognized in diverse taxa and is generated by the metabolic rate (White *et al.*

2007). The negative relationship between body size and abundance, which is acquired by tracking communities through time, is called cross-community scaling relationship (CCSR). When habitat resource availability is stable through time, body size directly determining abundance is the regular pattern in CCSR as discussed by White and his colleagues. But our results supported the inverse effects of rapidly increasing abundance on significantly decreasing body size in freshwater crabs. In order to reduce extinction risks of extreme catastrophes, freshwater crabs rapidly increased their abundance with relative stable habitat resource, and thus led to the"side-effect" of body size decline in short-term study period.

Although we found the trend pattern of their rapidly increasing numbers to counteract surviving threats, specific species such as *Geothelphusa shernshan* and *G. lili*, which were restrictedly distributed in affected area with small populations, still decreased in population abundance and suffered much higher extinction risks than the others after Typhoon Morakot (unpublished data).

We depicted the adaptation strategy and the different responses of freshwater crabs and their habitats in short-term study period after extreme catastrophes, but establishing long-term datasets are important for biodiversity research (Magurran *et al.* 2010). It is crucially important for the rare, endangered (Cumberlidge *et al.* 2009) and totally endemic (Shih and Ng 2011) resource of freshwater crabs in Taiwan. Long-term monitoring, which gauges not only the background data of specific species but also the variation patterns of the ecosystem, will provide critical information for conservation policy. We continue to monitor freshwater crabs and their habitats in important study sites to collect long-term datasets.

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醋酸甲羥孕酮及左炔諾孕酮利用於圈養台灣獼猴生殖控制之研究

The effects of medroxyprogesterone acetate and levonorgesterel in the reproductive control of captive *Macaca cyclopis*

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摘 要

台灣獼猴(*Macaca cyclopis*)爲台灣特有種，近來隨著棲地開發及族群擴張，其與人類生活空間重疊情形日增，並衍生許多衝突。本研究目的以簡單實用與非侵入性的操作方式控制臺灣獼猴的動情週期，期望可以達到抑制臺灣獼猴繁衍過度之情形。本研究將成熟雌性之臺灣獼猴分爲三組：第一組使用醋酸甲羥孕酮(medroxyprogesterone acetate)以肌肉注射方式給予；第二組左炔諾孕酮(levonorgestrel)以口服投予方式投藥；第三組爲對照組。試驗進行前獼猴先依血漿中孕酮(progesterone)與雌二醇(estradiol)濃度、陰道抹片及性皮腫脹觀察動情週期的變化。於非交配季節觀

察雌性獼猴之動情週期平均爲 28.29 ±5.3 天，陰道細胞學與排卵週期具有週期性變化，但性皮腫脹程度及增紅程度並不明顯。試驗結果發現：第一組獼猴使用醋酸甲羥孕酮注射後 11 週，其血漿中孕酮濃度達到最高峰平均爲 281.42 ±51.18 ng/mL，顯著高於對照組與正常動情週期黃體期之濃度，但雌二醇變化不受影響，顯示試驗獼猴仍處於黃體階段。第二組獼猴使用左炔諾孕酮口服投予兩次後，口服後第 7 週其血漿中孕酮濃度到達最高峰平均值爲 253.70±50.11 ng/mL，顯著高於對照組表示可造成延遲排卵。試驗可知臺灣獼猴以肌肉注射醋酸甲羥孕酮可成功延長成熟雌猴之動情週期與排卵至少三個月，較適合做爲野外獼猴非侵入性生育控制方法。

Abstract

This study was undertaken to develop a simple and practical method to control the time of ovulation in *Macaca cyclopis*. Group 1 (*n*=6) was treated with a single 150 mg intramuscular injection of medroxyprogesterone acetate (MPA) injectable suspension, while Group 2 (*n*=5) was treated with diets containing 0.3 mg levonorgestrel (LNG) given weekly to normally cycling female monkeys for 2 weeks, and Group 3 (*n*=3) remained untreated. Plasma concentrations of estradiol and progesterone, vaginal smear and conspicuous swellings were determined in order to estimate the estrus cycle. The average length of the normal menstrual cycle in the non-mating season was 28.29 ±5.3 days. Vaginal smear, were observed in relation to ovulation. However, conspicuous swellings of the perineal skin and timing of ovulation are not obvious in females of *Macaca cyclopis* particularly during the middle stages of the ovarian cycle. Group 1 treated with MPA, ovulation occurred more than 120 days after medication. MPA administration resulted in a significant increase in plasma progesterone levels 11 weeks after medication (281.42 ±51.18 ng/mL) and plasma estradiol values did not differ significantly in all monkeys. Group 2 was treated with LNG at 0.3 mg/weekly for two weeks, plasma progesterone level was significantly greater (253.70 ±50.11 ng/mL) than those in the control group and the results indicated that ovulation occurred more than 28 days after the last administration in all monkeys. The results indicate that a single 150 mg IM dose of MPA could be applicable for delaying ovulation, and suggest that MPA is a more proper way of non-invasive reproductive control for the wild *Macaca cyclopis*.

關鍵字：臺灣獼猴、生殖控制、醋酸甲羥孕酮、左炔諾孕酮

Key words: *Macaca cyclopis*, reproductive control, medroxyprogesterone acetate, levonorgestrel

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緒　言

臺灣獼猴(*Macaca cyclopis*)在分類上屬於靈長目(Primates)、獼猴科(Cercopithecidae)、獼猴屬(*Macaca*)，爲臺灣特有種，日行性且爲群居生活的野生動物。對環境的適應性強，從平地到海拔 3,600 m 間的各類林地中皆可發現，但以 500 至 1,500 m 的闊葉林較爲常見 (鹿野 1929；Kuroda 1940；McCullough 1974)，分佈範圍主要集中在中央山脈與海岸山脈地區的各個鄉鎮，估計全島臺灣獼猴約 10,404 群(李等 2002)。猴群大小由數隻到數十隻不等，但一般以 20 至 30 隻爲多，且有隨著海拔升高而下降的趨勢。以調查所得平均猴群個體數 25 隻推算，全島臺灣獼猴數量約有 25 萬隻。

臺灣獼猴之動情週期相關研究方面，Peng 等人於 1973 年觀察爲 29.4 ±15.5 天(Peng *et al.* 1973a)，Chen 等人於 1990 年觀察結果爲 27.8 ±2.9 天(Chen *et al.* 1990)，季等人於 1997 年研究則爲 27.3±8.9 天(季等 1997)，由上述結果得知，成熟之雌性臺灣獼猴如同人類一樣具有動情週期，但與人類不同的是，臺灣獼猴具有顯著交配季節，臺灣獼猴的交配季(Mating season)爲每年的 9 月至隔年的 2 月，生育期(Birth season)爲每年的 4 月至 6 月(Hsu *et al.* 2000)，妊娠期(Gestation period)約爲 162 天(Peng *et al.* 1973a；Peng *et al.* 1973b)。臺灣獼猴適應力強，原本從平地到高山到處可見牠們的蹤跡且數量不斷的在增加與分群，外加氣候的變遷與人爲開墾與濫伐等破壞了臺灣獼猴的棲息地等，使得臺灣獼猴危害農作物及攻擊登山客的事件始終受到媒體的關注。

醋酸甲羥孕酮 (Medroxyprogesterone acetate；MPA) 是一種人工合成的孕激素，爲黃體酮衍生物，作用類似於黃體酮，但孕激素作用較強，無雌激素作用(Schindler *et al.* 2003)，全球約有五億人使用它作爲避孕用途(Morrison *et al.* 2012)。 MPA 可做成口服藥錠或是肌肉注射及皮下注射等劑型，因藥物爲微結晶狀態，因此可以在注射之後從組織中緩慢釋放，而有較長的作用時間(Mishell 1996)。MPA 作用於子宮內膜，促進黏膜的增殖分泌，通過對下視丘的負迴饋，抑制促黃體激素、促腎上腺皮質激素及其他生長因素的釋放，使卵泡不能發育成熟，抑制卵巢的排卵過程(Mishell 1996)。

MPA 在非人類靈長類的應用方面，Mora 等人對四隻恆河猴(*M. mulatta*)注射同樣劑量的 MPA，並檢測後續血液中 MPA、雌二醇及助孕素的濃度，發現其曲線與成年婦女相似(Mora *et al.* 1976)。Hild-Petito 等人對八隻恆河猴給與 MPA 皮下注射 30mg 後，於 10、30、60、118 及 146 天進行陰道生檢，檢查陰道上皮細胞的厚度、數量和型態，比較給藥前後的生檢結果，發現給藥後的結果與給藥前濾泡期及黃體期的結果沒有顯著差異，此類藥物主要會造成陰道上皮細胞變薄，並且在療法結束後能很快回復，而能夠順利控制生殖週期(Hild-Petito *et al.* 1998)。另外在其他種類的獼猴方面，Shimizu 對五隻食蟹獼猴(*M. fascicularis*)皮下注射 15 mg/kg 的 MPA，能夠產生抑制生殖週期及排卵長達 161±17 天的效果；Shimizu 同時對一群自由放養的日本獼猴(*M. fuscata*)皮下注射 MPA，第一年對 15 隻雌性日本獼猴施打劑量 7.5mg/kg，仍然有交配以及產子現象，隔年更改劑量爲 19.4mg/kg，對同樣族群的五隻雌性獼猴施打，受試的獼猴在當季便沒有交配行爲，也未產子(Shimizu 1996)。

左炔諾孕酮(Levonorgestrel；LNG)屬於人

工合成孕激素分類中的 19-Nortestosterone 衍生物類(Schindler *et al.* 2008)。LNG 廣泛的使用於臨床高效合成避孕藥，於臨床上有口服製劑、子宮內節育器、皮下埋植劑和靜脈注射形式以供使用，爲目前最理想的避孕藥之一(Moore *et al.* 1995; Munro *et al.* 1996)。其避孕之作用機轉爲：使子宮內膜腺體數量變少、腺體直徑縮小不利於受精卵著床，在性行爲發生後 3-10 小時內給予 0.4mg 會在 3 小時後下降精子在子宮腔中的數量、在 5 小時後使子宮頸黏液鹼化使精子活動下降、在 9 小時後子宮頸黏液黏滯度上升，在體內黃體激素濃度到達高峰前給予會抑制促性腺激素分泌而使動情素、孕激素、子宮頸黏液的分泌下降以及降低性荷爾蒙結合球蛋白的數量(Landgren *et al.* 1994)。LNG 在靈長類的使用結果依種別與方法不盡相同，在某些研究中確實指出了他有延遲排卵的功能。在 Nagle 等人的研究中，發現在排卵前 8 小時以上使用相隔 8~9 小時，兩次 2mg 的口服 LNG，可以抑制卷尾猴(*Cebus capucinus*)的卵從濾泡中釋放，若是已經排卵則沒有效果(Nagle *et al.* 2009)。在食蟹猴也有類似的研究報告，若每天將 50μg 的 LNG 混在飼料中，可以延長動情週期的長度，但相同的概念也一樣在這個研究中發生，在動情週期中(25–35 days)的 16-29 天使用比起在其他階段使用更可以有效的延長動情週期(Nakama *et al.* 2012)。另外，由 Ortiz 等人所作的研究更明確的顯示使用 LNG 時間點的重要性，他們指出，卷尾猴(*Cebus apella*)已經交配過後 1 小時內，使用口服或是皮下注射 0.75 mgLNG，無法影響其懷孕率。雖然有抑制排卵的功能，但也僅限於濾泡大小不超過 5mm 的階段(Ortiz 2004)。除了口服給予，聖路易斯動物園做了一些有關 LNG 的使用研究，利用皮下的方式將含有 70mgLNG 的小管植入白面僧面猴(*Pithecia pithecia*)皮下，可以完全地阻止排卵至少 8 個月(Savage *et al.* 2002)。在絨頂檉柳猴(*Saguinus oedipus*)中，利用含有 LNG 的膠體打入皮下，也有類似的結果(Wheaton *et al.* 2010)。還有另外一種運用方式是將覆蓋 LNG 的子宮內避孕器放入恆河猴的子宮內，每天釋放 25μg，可以影響到子宮腺體的發育以及基質的厚度，對動情週期也有一定程度的影響(Wadsworth *et al.* 1979)

綜合上述，使用注射 MPA 或口服 LNG 之方式在靈長類的動物能夠有效發揮藥物特性，藉由抑制排卵及延長動情週期之方式達到生殖控制的效果，然而相關之研究在台灣獼猴尚呈闕如，因此本計畫研究動機與目的將針對臺灣獼猴建立初步之動情週期資料，並且以口服 LNG 或肌肉注射 MPA 方式，測試其影響動情週期之情形，藉此推估適合給藥時間。利用此種非侵入式方法改變臺灣獼猴動情週情，以討論獼猴生殖控制之可行性。

材料及方法

本研究利用飼養於南投縣政府名間苗圃(南投縣名間鄉大老巷 101 號)6 - 15 歲之臺灣獼猴 16 隻，其中包含雌猴 14 隻及誘發雌猴發情之雄猴 2 隻，所有獼猴均單一籠飼於不鏽鋼保定籠(100 cm × 76 cm × 76 cm)內，每天餵飼兩餐，包含水果與猴科飼料等食物。雖所有雌猴均爲性成熟母猴，爲確保各年齡層之雌猴平均分配於各組，雌猴依體重、牙齒、外觀進行分組：第一組 6 隻以肌肉注射長效型 MPA(Depo-Provera®, Pfizer, Kalamazoo, USA)進行試驗；第二組 5 隻以口服方式投予 LNG(Microgynon 30, Bayer Schering Pharma

AG, Weimar, Germany)進行試驗，投予時將LNG 錠劑加入食物中餵食，並確定完全食入；第三組共 3 隻作爲對照組，持續監測發情週期之血液中的孕酮與雌二醇濃度。實驗期間自民國 101 年 12 月至 102 年 7 月止共進行 8 個月的時間，試驗獼猴於移籠後每個月月底進行一次抽血健康檢查，共監測四個月期間，再進行分組監控與給藥計畫如下。

一、建立臺灣獼猴之動情週期資料庫：

受試母猴於實驗期間每隔 5-7 日進行一次健康檢查，母猴於大腿外側肌肉注射 4 mg/kg Zoletil® 50(Zoletil®, Virbac, Carros, France)，於 30 - 50 分鐘的麻醉時間內進行外觀觀察、血液樣本採集及陰道抹片檢查的工作。

外觀觀察乃針對雌猴之性皮腫脹與否進行觀察及描述。

血液樣本採集是以 23 號頭皮針，於後肢靜脈採集約 3 - 4 mL 的血液，分別進行荷爾蒙濃度檢測及健康檢查：檢測血中孕酮與雌二醇濃度則將採得 2 mL 血液經 6,000 g，10 分鐘離心後，取血清分裝於離心管於-80 ℃保存已備分析之用。待測樣本分別使用商業化套組 Cayman Chemical® Progesterone EIA Kit (Cayman Chemical company, Ann Arbor, USA) 與 Cayman Chemical® Estradiol EIA Kit(Cayman Chemical company, Ann Arbor, USA)檢測血中孕酮與雌二醇濃度。操作流程如產品資訊。(https://www.caymanchem.com/ pdfs/582601.pdf, https://www.caymanchem.com /pdfs/582251.pdf)

陰道抹片檢查則是以沾生理食鹽水之棉花棒直接插入陰道內輕輕移動且轉動，取出後直接塗抹於載玻片上再以劉氏染色法(Liu's stain A and B, ASK®, Taipei, Taiwan)染色隨後將玻片烘乾，以二甲苯封片後，利用光學顯微鏡觀察陰道上皮細胞的變化。

二、不同避孕藥劑影響其動情週期之情形：

將三組雌猴分別做下述利用：第一組 6 隻雌猴進行肌肉注射 MPA 進行試驗，劑量爲 150 毫克/次，投予一次，利用酵素免疫分析法檢驗血液中的孕酮與雌二醇濃度變化。第二組之 5 隻雌猴進行口服方式投予 LNG 進行試驗，劑量爲 0.3 毫克/次/週，投予時間兩週，利用酵素免疫分析法檢驗血液中的孕酮與雌二醇濃度變化，分析其影響動情週期之效果與效期。第三組之 3 隻雌猴作爲對照組並給予口服投與安慰劑(維他命)與注射生理鹽水，三組於投藥後持續以每 5 至 7 日抽血一次之頻率監測血液中的孕酮與雌二醇濃度，分析其影響動情週期之效果與效期。

三、藥物安全性：

從投藥前四個月開始取每月麻醉採集之血樣進行健康檢查並做爲投藥後每月採集之血液檢查結果之比較基準，同時與台灣獼猴之血液學參考值比較(李，1994)以做爲藥物對於受試獼猴整體健康狀況影響之評估。檢驗項目包含(一)全血球計數：採得 0.5 mL 血液注入含有乙二胺四乙酸 (ethylenediaminetetraacetato；EDTA)的眞空採血管中，上下均勻混合血液後，取適量血液利用全自動血球分析儀(Medonic CA530 Vet 9 Parameter System, Boule Medical AB, Stockholm, Sweden)進行紅血球總數、血容比、血紅素、平均紅血球血紅素、平均紅血球血紅素濃度、血小板及白血球項目分析。(二)血清生化學與血中離子濃度檢驗：採得 1.5 mL 血液注入含有肝素(heparin)的眞空採血管中，上下均勻混合血液後，將血液經 6,000g，10 分鐘離心後，取 1 mL 血清分裝於檢體杯中，分別利用自動血清生化分析儀(Hitachi 705 Automatic Analyzer, Boehringer

Mannheim Diagnostic, Houston, USA)檢測鹼基磷酸酶、天門冬胺酸轉胺酶、丙胺酸轉胺酶、總膽紅素、血尿素氮、總膽固醇、肌酸酐、肌酸激酵素酶、血糖、磷、總蛋白質、乳酸去氫酵素、谷氨酰轉肽酶等項目並以血中離子濃度分析儀(Biolyte 2000 Na^+, K^+, Cl^-/Li^+ Electrolyte Analyzer, Biocare corporation, Taoyuan, Taiwan)進行分析血中鈉、鉀、氯離子濃度。

四、統計分析：

使用 SPSS 進行統計分析。以 paired *t*-test 比較注射及口服前後雌二醇及孕酮差異性，若 $p < 0.05$ 顯示兩組數據之間具顯著差異，若 $p < 0.01$ 則顯示具極顯著差異。

結　果

臺灣獼猴動情週期之內泌素與陰道細胞學變化

試驗開始後實驗雌性臺灣獼猴(n=14)由血液與血清生化學的數據判斷，試驗動物仍處於輕微脫水、緊迫與適應環境階段，因此動情週期內分泌素變化並不明顯，若以三次動情週期觀察時，其動情週期平均爲 28.29 ±5.3 天，若以正常飲食與環境適應後進行觀察，於連續二次正常動情週期(n = 14)的結果發現，動情週期平均爲 28.04 ±3.0 天(表 1)

表 1. 14 隻成熟雌性臺灣獼猴依陰道抹片及外陰部觀察所得之連續三次動情週期

Table 1. Three consecutive reproductive cycles of mature female *Macaca cyclopis* based on vaginal swab and appearance

No.	1st (days)	2nd(days)	3rd(days)
F03	37	29	27
F04	37	27	32
F05	37	27	27
F06	19	29	27
F07	35	34	27
F08	16	29	29
F10	29	34	32
F11	37	22	29
F12	29	27	29
F13	22	27	24
F14	22	27	29
F15	15	22	32
F17	34	29	24
F18	34	27	27
average	28.79	27.86	28.21
STD	8.34	3.44	2.61

使用商業化套組偵測對照組血液中雌二醇與孕酮濃度；在雌二醇部分，其血液中濃度介於 118.78±22.70 pg/mL-224.33±8.76pg/mL 間循環，每 4 週爲一個週期(圖 1)；孕酮部分，則介於 97.51±19.83ng/mL-161.88±26.84 ng/mL 間，且與雌二醇相同，皆爲 4 週一個循環(圖 2)。

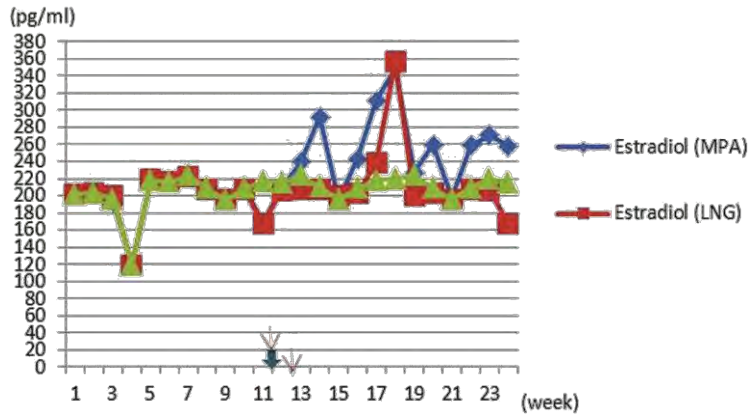

圖 1. 受試台灣獼猴之雌二醇變化情形，實線箭頭爲 MPA 投藥時間；虛線箭頭則爲 LNG 投藥時間。由圖可見三組雌猴之雌二醇於投藥前具極高之相關性，並且每四週出現一次高峰。投藥後 MPA 組之雌二醇有統計上不顯著之升高但仍與對照組同時維持四週一次高峰之週期；但 LNG 組則於第 13 週未見雌二醇之上升且延遲至第 17 週才上升顯示有延後發情之情形。

Fig. 1. Weekly estradiol concentrations of *Macaca cyclopis* from different treatment groups. There is high consistency between three groups before treatment and 4-weeks cycle is prominent. After medication, the estradiol concentration of MPA group had insignificant elevation but still poses 4-weeks cycle as the control group. However, the LNG group had delayed elevation until week 17, which showed delayed estrus.

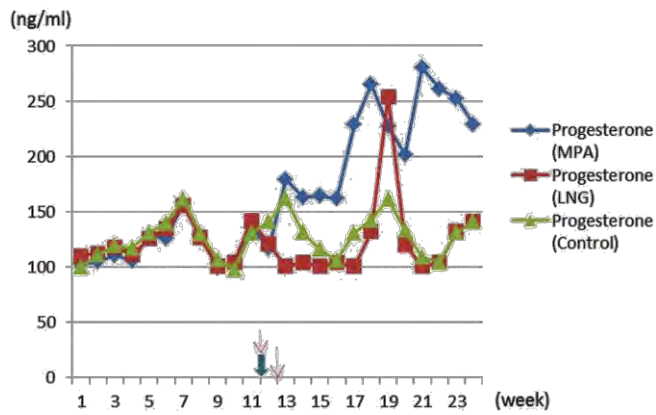

圖 2. 受試台灣獼猴之孕酮變化情形，實線箭頭爲 MPA 投藥時間；虛線箭頭則爲 LNG 投藥時間。由圖可見三組雌猴之孕酮於投藥前具極高之相關性，並且每四週出現一次高峰。MPA 組於投藥後孕酮即開始上升且至第 23 週仍維持高值，顯示獼猴處於黃體期；而 LNG 組則於第 13 週未出現應有的高峰且延至第 19 週才出現，顯示有延後發情的情形。

Fig. 2. Weekly progesterone concentrations of *Macaca cyclopis* from different treatment groups. There is high consistency between three groups before treatment and 4-weeks cycle is prominent. After medication, the progesterone concentration of MPA group had gradual elevation until week 23, which showed the females are in the luteal phase. However, the LNG group had delayed elevation until week 19, which showed delayed estrus.

於麻醉採血的同時進行陰道抹片檢查，利用棉棒刮取陰道上皮塗抹於玻片上，且利用劉氏染色進行細胞染色，使用顯微鏡觀察。觀察發現性成熟的雌猴其陰道上皮與犬貓等哺乳動物類似，可分成三種不同的上皮細胞：基底細胞區(basal zone)、中間層細胞區(mid zone)，及角化鱗狀上皮細胞。綜觀此三層細胞隨動情素的濃度消長亦與其他哺乳動物相似：動情時陰道抹片可見多量的白血球與紅血球，而未完全角化之中間層細胞數量較多，角化鱗狀上皮細胞數量較少(圖 3)，至濾泡期角化細胞增加(圖 4)，在排卵期間，角化鱗狀上皮細胞達到最高點，於鏡檢下可見大量核濃縮之角化上皮細胞(圖 5)，此時動情素之濃度也達到最高峰。於黃體期表層細胞漸減，而中間層細胞漸增，此時動情素濃度快速下降，並伴隨黃體素的上升，至動情前可見白血球再次出現(圖 6)。而投藥後內泌素與陰道抹片之細胞相間的相關性則敍述於下文。

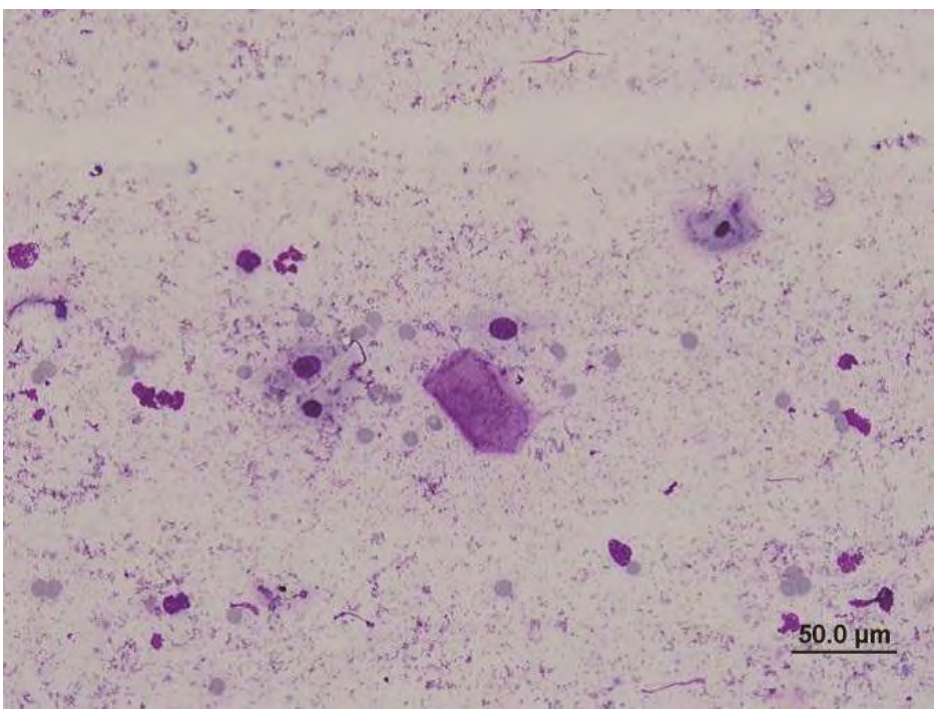

圖 3. 月經期，可見多量的白血球與紅血球，而未完全角化的中間層細胞較多，角化鱗狀上皮細胞較少。(Liu's stain, 400X)

Fig. 3. Menstrual phase. Marked white blood cell and red blood cell could be seen. Uncornified mid zone cells are more than cornified squamous epithelium cells. (Liu's stain, 400X)

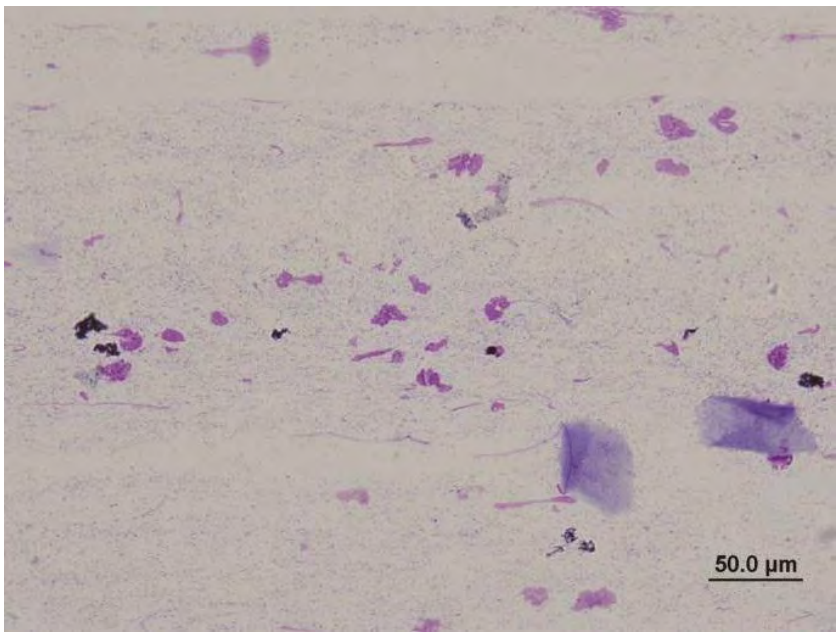

圖 4. 濾泡期，角化之表層細胞增加，未見紅血球及白血球。(Liu's stain, 400X)

Fig. 4. Follicular phase. There are increase amount of cornified squamous epithelium cells but without red blood cells and white blood cells. (Liu's stain, 400X)

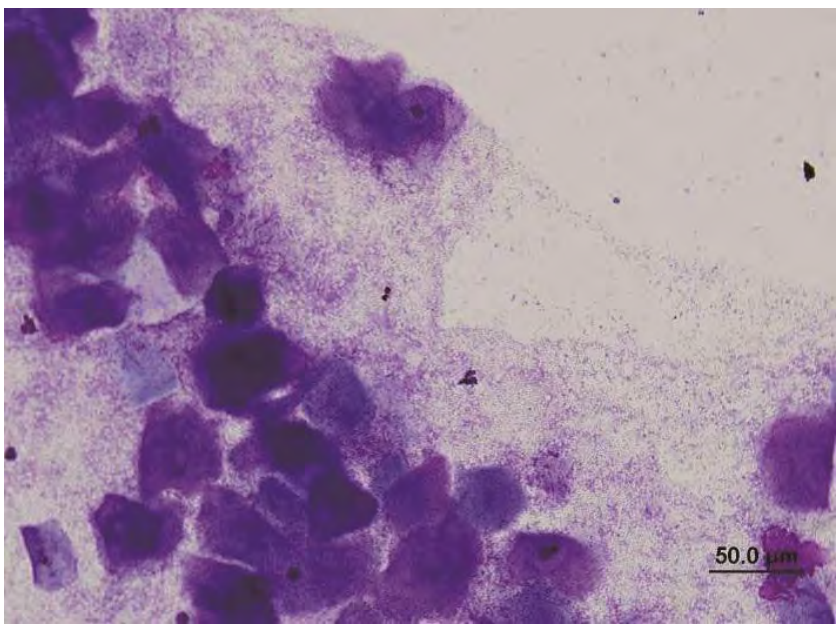

圖 5. 排卵期，表層細胞數量達到最高峰，鏡檢下可見大量核濃縮之角化上皮細胞。(Liu's stain, 400X)

Fig. 5. Ovulation phase. Epithelium cells peak at this stage. Large amount of cornified squamous epithelium cell with condensed nucleus could be seen microscopically. (Liu's stain, 400X)

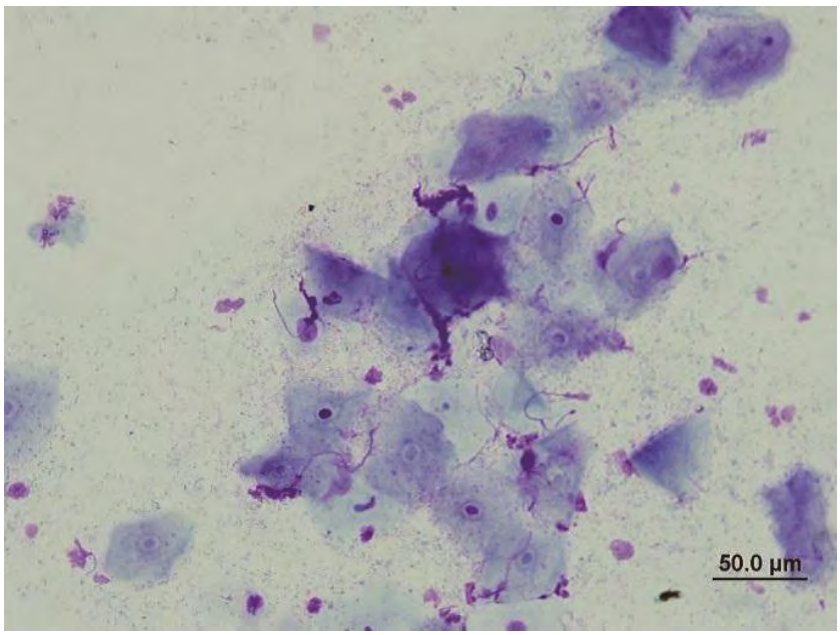

圖 6. 黃體期，表層細胞漸減，而中間層細胞漸增，至月經前可見白血球再次增加。(Liu's stain, 400X)
Fig. 6. Luteal phase. The number of epithelium cell decreased and replace by mid zone cells. The number of white blood cells will increase again before menstrual phase. (Liu's stain, 400X)

藥物給予後內泌素與動情週期變化

6 隻雌性臺灣獼猴以肌肉注射 MPA 進行試驗，劑量爲 150 毫克/次，投予一次後，試驗獼猴每週進行採血並以酵素免疫分析法檢驗血液中的雌二醇與孕酮濃度變化。結果發現注射後孕酮的變化由 103.96 ±27.59 ng/mL 每週顯著的上升至注射後第 7 週與第 8 週，分別爲 228.67 ±26.88 ng/mL 與 265.22 ±35.04 ng/mL，至注射後 11 週孕酮濃度上升至 280.18 ±21.67 ng/mL，皆與注射藥物前之變化與對照組具有顯著性升高($p< 0.01$)，且於注射後 14 週孕酮濃度仍爲 229.10 ±20.13 ng/mL($p < 0.01$)，代表試驗臺灣獼猴仍處於黃體階段(圖 2)。而雌二醇的濃度變化於注射前爲 209.33 ±5.86 pg/mL，注射後雌二醇濃度則介於 168.67 ±62.69 pg/mL 至 349.43 ±27.81 pg/mL(無顯著性差異)，每四週固定的週期性變化，對於投予之 MPA 並未產生影響(圖 1)。

陰道細胞學的變化則發現注射 MPA 後，試驗臺灣獼猴($n = 6$)皆處於黃體期與濾泡期階段，顯微鏡檢查主要均以中間層細胞與角化之表層細胞爲主，無明顯動情角化之變化。會陰部之性皮與顏色則無明顯腫脹與增色的改變。

另一組處理組於實驗開始第 11 週及第 12 週針對 5 隻雌猴以口服方式投予 LNG 進行試驗，劑量爲 0.3 毫克/次/週，投予時間 2 週，利用酵素免疫分析法檢驗血液中的孕酮與雌二醇濃度變化。口服 LNG 其孕酮與雌二醇之血清濃度檢測結果顯示，雌二醇於口服後第 5 週時開始爬升由給藥前 207.84 ±5.14 pg/mL 爬升至 238.60 ±35.71 pg/mL($p < 0.05$)，於口服後第 6 週時達高峰 357.32 ±65.70 pg/mL，於口服後

第 7 週(p＜0.01)下降至 199.40 ±34.75 pg/mL，並維持至口服後第 10 週輕微上升(圖 1)；孕酮部分，則至口服後第 7 週才由給藥前的 104.16 ±30.84 ng/mL 上升至 253.70 ±50.11 ng/mL(p＜0.01)，且於口服後第 8 週時下降至 120.20 ±12.26 ng/mL，並維持至口服後第 11 週開始緩慢爬升(圖 2)。陰道細胞學的變化則發現口服方式投予 LNG 後，試驗臺灣獼猴(n =5)皆處於黃體期階段，顯微鏡檢查主要均以中間層細胞爲主，且無觀察到明顯的性皮腫脹與顏色改變。

藥物安全性及臺灣獼猴健康檢查數值變化

針對本次實驗之雌猴進行稱重，投藥前體重爲 4.06 ±0.76 Kg，而投藥後的最後一次檢查之體重爲 4.78 ±0.99 Kg，兩者無顯著差異；而在投藥後全血球計數結果則如下：紅血球 5.11 ±0.53 × 10^6/μL，血容比 34.16 ±3.13 %，血紅素 11.73 ±1.04 g/dL，平均紅血球容積 67.01 ±3.66 fL，平均紅血球血紅素 23.07 ±1.53 pg，平均紅血球血紅素濃度 34.43 ±1.34，血小板 428.32 ±75.83 × 10^3/μL 與白血球 13.30 ±5.67 × 10^3/μL。此外，檢測血清生化學結果如下：ALR 4.67 ±0.53 IU/L，鹼基磷酸酶 314.20 ±211.91 IU/L，天門冬胺酸轉胺酶 47.73 ±28.47 IU/L，丙胺酸轉胺酶 101.17 ±84.98 IU/L，總膽紅素 0.35 ±0.14 mg/dL，血尿素氮 26.24 ±8.07 mg/dL，總膽固醇 135.93 ±32.94 mg/dL，肌酸酐 1.16 ±0.24 mg/dL，肌酸激酵素酶 85.00 ±40.56 IU/L，血糖 56.46 ±29.31 mg/dL，磷 3.96 ±1.00 mmol/L，總蛋白質 8.19 ±1.01 g/dL，乳酸去氫酵素 427.94 ±145.04 mmol/L 與谷氨酰轉肽酶 77.40 ±36.30 IU/L。雌猴檢測血中離子濃度結果如下：鈉 141.41 ±9.25 mmol/L，鉀 3.71 ±0.29 mmol/L 與氯 107.50 ±8.34 mmol/L。

討　論

臺灣獼猴之動情週期相關研究方面，Peng 等人於 1973 年觀察爲 29.4 ±15.5 天(Peng *et al.* 1973a)，Chen 等人於 1990 年觀察結果爲 27.8 ±2.9 天(Chen *et al.* 1990)，季等人於 1997 年研究則爲 27.3 ±8.9 天(季等，1997)，Nigi 等人觀察日本獼猴動情週期爲 26.3 ±5.4 天 (Nigi 1975)、Dukelow 等人觀察食蟹猴 30.9 ±4.8 天 (Dukelow 1975)。而本次的試驗研究中發現，試驗臺灣獼猴經長途運輸、緊迫、適應環境與輕度脫水等情況導致第一次動情週期徵候並不明顯，第一次完整動情週期平均爲 28.79 ±8.34 天，顯示個體間差異較大，飼養約一個月後，因適應新的環境與充足食物及飲水後，第二次與第三次動情週期則趨於穩定，平均分別爲 27.86 ±3.44 天與 28.21 ±2.61 天，個體間差異逐漸減小，與前人研究之結果亦爲相似。此外試驗獼猴爲籠飼飼養模式，因此以麻醉方式每週進行採血，而依據前人研究，多次的麻醉及靜脈抽血不會對性週期造成任何有意義的改變(Hotchkiss *et al.* 1971; Stabenfeldt and Hendrickx 1973; Channing *et al.* 1977; Aidara *et al.* 1981)。

於試驗進行觀察臺灣獼猴正常動情週期時其性皮腫脹的變化，結果其腫脹及增紅程度並不明顯，不似黑猩猩與狒狒般有明顯性皮腫脹 (Wildt *et al.* 1977; Deschner *et al.* 2004)，本次試驗結果與季等人之研究相似，認爲臺灣獼猴雌猴在交配季中會有外觀變化，但變化的部分及程度，會因個體而有所差異，或是性皮的腫脹及增紅，或是臉部膚色的增紅，也有少部分個體甚少有外觀的變化。性皮腫脹增紅期，多在經血前或後半個月，而此時與雄猴間的交配及配對關係亦較頻繁 (季 1996)。因此，若

於野外進行試驗時，以性皮腫脹變化程度作爲動情週期的觀察指標可能會有所差異。

於本次試驗中，陰道細胞學檢查若能降低糞便汙染與進行劉氏染色時非特異性結合的染色顆粒外，可順利以顯微鏡方式觀察以作爲卵巢週期性變化的佐證，若同時配合內分泌素(孕酮與雌二醇)的分析下，可以充分掌握正確的排卵時間與動情週期。

針對高雄壽山地區進行臺灣獼猴生殖行爲研究中發現，該地區臺灣獼猴交配高峰期爲11 月至 12 月(黃 2003)，此與季等人(1997)及李等人(2002)對圈養台灣獼猴觀察結果相似。因此，配合本實驗結果，MPA 對於試驗成熟雌性臺灣獼猴能夠維持三個月以上的黃體期，成功降低其發情頻率與有效延長動情週期，且以肌肉注射之給藥途徑與藥物半衰期長等均爲 MPA 適用於野外工作的優點。因此，每年 8 至 11 月間於野外使用 MPA 進行避孕，應可順利達到抑制臺灣獼猴繁衍過度之情形。

Nakama 等人於 2012 年發表文獻表示給予口服避孕藥物(LNG)，可有效延長排卵週期，由原先 16 - 29 天，延長 26 至 36 天 (Nakama *et al.* 2012)，而根據本實驗之結果顯示，在給予口服 LNG 後，不論雌二醇(口服後第 5 週)或孕酮(口服後第 7 週)均延遲 4 週才上升；顯示口服給於 LNG 確實能有效延長排卵週期；然而根據本次實驗在口服後第 10 至第 12 週之結果中，雌二醇與孕酮同時出現上升之情形，顯示口服給與 LNG 雖能達到延長排卵週期，然而藥效過短，若想有效延長排卵週期，則需於每次排卵週期開始時皆給予口服 LNG。因此於田間以口服方式投予 LNG 的方式可能必須以密集的投予方式，但又未能確保該隻臺灣獼猴可以連續性食入此藥，因此若以此藥進行田間試驗可能必須改變其投藥方式。

由李於 1994 年檢測臺灣獼猴之血液學與血清生化學正常值與本次實驗中的檢測值比較可得知：在全血球計數中，血容比略低於前次研究，白血球數值高於前次研究；而在血清生化學方面，其數值範圍則均落於參考值範圍內，同時由體重資料也未觀察到明顯波動，由此可知，兩藥物於獼猴之使用並未見到血清生化學上的副作用，也沒有於人類使用常見的如食慾不振、體重起伏等副作用。

綜合上述，藉由藥物進行台灣獼猴之生育控制是安全有效的方法，且以 MPA 較爲可行，然而野外工作的進行，尚需配合野生族群之發情週期及分布狀況設計最佳的投餌時機及數量以達到最大效率，而此尚仰賴更進一步且大規模的野外研究。

謝　誌

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阿里山根節蘭根菌結合體之微細構造及內生菌

Ultrastructure and Endophytes of Root-Fungus Association of *Calanthe arisanensis*

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摘 要

阿里山根節蘭爲臺灣特有種植物；本研究所採之樣本在根菌結合體中所誘導純化之 4 株內生菌，經分子生物鑑定分別應屬於 Sordariomycetes、*Neonectria* 和 *Xylaria* 菌株。另於根菌結合體可觀察到腫大及其表面有稠密的菌絲覆蓋；利用電子顯微鏡進行微細構造觀察，發現在內皮層有菌絲團及老化的菌絲團。因此，本研究結果證實，南投縣郡大林道之阿里山根節蘭之根菌結合體具有蘭花菌根之構造。

Abstract

The objectives of this study were to investigate the root-fungus association characteristics of *Calanthe arisanensis*, an endemic species in Taiwan. The isolated fungal strains from *Calanthe arisanensis* have been identified as Sordariomycetes, *Neonectria* and *Xylaria* by a DNA sequence analysis. The root-fungi associations from the site were found swollen and densely adhered to the surface by hyphae under a stereomicroscopy. Young and old peloton structures in the root cortex of *Calanthe arisanensis* were observed with a scanning electron microscope. These results demonstrate that *Calanthe arisanensis* could form orchid mycorrhiza in the field.

關鍵詞：內生菌、阿里山根節蘭、蘭花菌根、微細構造

Key words: *Calanthe arisanensis* Hayata, endophyte, orchid mycorrhiza, ultrastructure

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前　言

臺灣蘭科植物根據臺灣植物誌第二版中共計記錄 101 屬 336 種，爲臺灣植物中包含最多物種的科（Su *et al.* 2000）；臺灣位於亞熱帶，正是蘭花生長最繁盛的地域，加上臺灣的地形錯綜複雜，海拔高差從海濱一直爬升到將近 4,000 m，在這其間包括熱帶、亞熱帶、溫帶和亞寒帶等多種氣候帶，使得臺灣的蘭花種類多樣，從保育物種多樣性的觀點來看，它應是不可忽視的一群。近年來，蘭花生育地受到破壞與物種遭受濫採等情形日漸嚴重，許多過去曾被記錄的物種，現已難覓其蹤跡（鐘等 2006）。

菌根（mycorrhiza）是自然界中普遍存在的植物共生現象，是由土壤中的眞菌與植物根系共生後所形成之一種根菌結合體（root-fungus association）(Frank 1885)。菌根形態共計有 6 種，主要分爲外生、內生及外內生菌根等三大類（弓 1997; Larcher 2003）。蘭花菌根（orchid mycorrhiza）係屬內生菌根（edomycorrhiza）；最早在 1840 年首由 Link 學者於蘭科植物根段內分離出內生眞菌（endophytic fungus）；之後，在 1886 年 Wahrlich 學者亦證實超過 500 種蘭科植物都具有內生眞菌（Arditti 1967）；而在國內也有多位學者針對臺灣原生蘭科植物之內生眞菌進行研究（陳 2007；鄭 2012）。

阿里山根節蘭(*Calanthe arisanensis*) 係屬蘭科(Orchidaceae)根節蘭屬(*Calanthe*) 中型地生蘭花植物，爲臺灣原生特有種，分布於全臺灣 1,000-2,000 m 的森林中；花色全白或有的微泛淡紫暈，花期是 2-3 月。

目前，雖然已有學者針對阿里山根節蘭之葉部進行化合物萃取，並其活性成分部分對於多種癌症細胞具有顯著毒殺作用(Lee *et al.* 2008)；而在蘭花菌根的研究上，尚無相關研究探討阿里山根節蘭之根菌結合體及其內生菌。本研究是以此臺灣特有種爲材料，進行菌根構造觀察，探討其根菌結合體是否具有蘭菌根之構造特徵；並觀察根系所分離菌株之多樣性。

材料與方法

I、材料

本研究樣本於春季採自於臺灣省南投縣郡大林道 15 km 處（東經 120°54′42.375″，北緯 23°37′51.083″）。

II、根系結合體形態觀察

利用自來水將野外採集的樣本流動清洗三分鐘後，置入超音波震盪機，再將根系結合體置於光學顯微鏡觀察下觀察形態（Usuki and

Narisawa 2005）。

將一部份樣本浸置於 2.5 % 戊二醛（glutaraldehyde）固定液中，固定一天，再以磷酸緩衝溶液（0.1M）清洗 3 次，每次 15 分鐘，隨後以酒精（10、30、50、70、80、90、95、100%）及 100 % 丙酮進行序列脫水，再以液態二氧化碳進行臨界點乾燥（CPD，Critical Point Drying）(陳等 1991)。隨後將材料以雙面膠黏於鋁台（stub）鍍金後，處理後利用掃描式電子顯微鏡進行觀察（蔡 2003）。

III、菌種分離與純化

將本次採集 5 株阿里山根結蘭之根菌結合體先以清水清洗後，利用立體顯微鏡進行觀察；再將 100 份根段樣本清洗後，置於無菌操作台上以 30% H_2O_2 溶液中殺菌 1 分鐘（Berch *et al.* 2002），再用無菌水清洗 3 次。隨後將清洗後的根段置於 1% water agar 培養基中（Xiao and Berch 1992）。置於 20℃ 黑暗環境下培養 14-21 天後，菌株會從根段邊緣長出，再將此菌絲移入 1% MEA（malt extract agar）培養基中進行純化（Johansson 2001）。

IV、菌落形態及生長觀察

將已純化之菌株，移至 2 % MEA 及 PDA 培養基上，在黑暗生長箱中培育（23℃），定時紀錄菌落生長及形態觀察（Hambleton and Currah 1997）。

V、菌種保存

將菌株移入 PDA 培養基試管中，進行菌種保存（蔡 2003）。

VI、DNA 萃取、PCR 擴增、序列分析和資料分析

將分離純化之菌株培養於 PDA 培養基，利用 Puregene DNA Purification Kit 來進行菌株 DNA 之萃取；利用引子 ITS1-F 和 TW13 來進行 ITS/5.8S rDNA 序列之聚合酶連鎖反應（polymerase chain reaction, PCR），再將純化後之 PCR 產物定序，並與 NCBI 網站資料進行序列比對，最後利用 MEGA 軟體繪製親緣關係樹狀圖（Sigler *et al.* 2005）。

VII、統計分析

將各菌株生長資料（3 重複），以變異數分析（analysis of variance，ANOVA）進行各處理間之顯著性分析，再以 Duncan 氏多變異檢定法進行同質性檢定。

結果與討論

I、野外採集菌根結合形態

在南投縣郡大林道所採集的阿里山根結蘭，其根菌結合體經攜回實驗室清洗乾淨後，利用光學顯微鏡觀察，可發現其植株僅有腫大的主根，並無其他細根（圖 1A）；另於其根菌結合體上有有稠密的菌絲覆蓋在表面（圖 1B）。

II、顯微構造

本研究在郡大林道所採集的阿里山根結蘭之根菌結合體，利用掃描式電子顯微鏡觀察時，在樣本表面可發現稠密菌絲覆蓋（圖 2A）；另在樣本橫切面上無發現菌毯（mantle）及哈替氏網（hartig net）等外生菌根的構造，但於此樣本之內皮層細胞內可發現菌絲團之構造（圖 2B），於菌絲團之構造中又可發現老化的菌絲團，此相鄰 2 個細胞間之老化菌絲團有菌絲相連之現象（圖 2C）。

依據菌根分類指出蘭花菌根係屬內生菌根之一(弓 1997; Larcher 2003)；然而，蘭花菌根的形態特徵係指在蘭科植物根系表皮細胞中菌絲會穿透細胞壁，並在皮層細胞內會出現初期及老化的菌絲團（young and old pelotons）構造(Hadley 1982; Chang and Chou 2007)。

III、菌株分離純化後菌落形態及生長觀察

本研究將分離之內生菌分別置於不同培養基（2%MEA及PDA）下培養（圖3及表1），觀察菌株在不同培養基下之形態變化，以作爲菌株鑑別之依據。

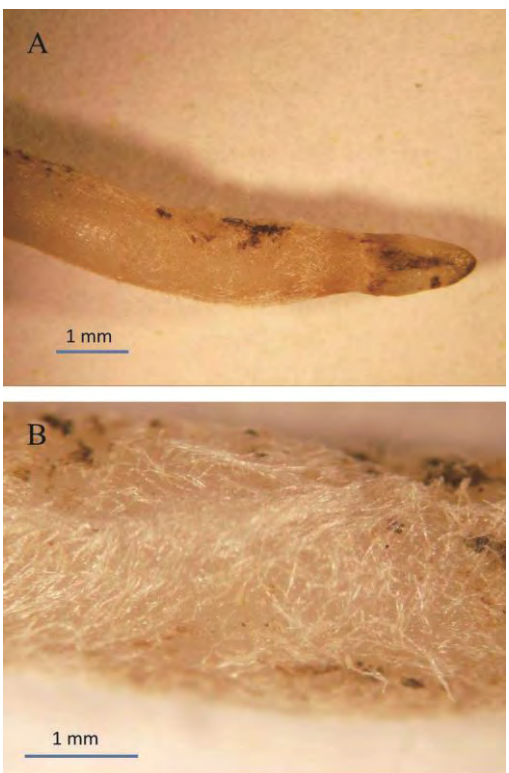

圖 1 阿里山根結蘭之根菌結合體形態。
A：腫大的主根(標尺=1mm)；B：表面有菌絲覆蓋(標尺=1mm)。

Fig. 1 Morphology of *Calanthe arisanensis* root-fungus association.
A: swollen association (bar＝1mm); B: association surface with hyphae (bar＝1mm)

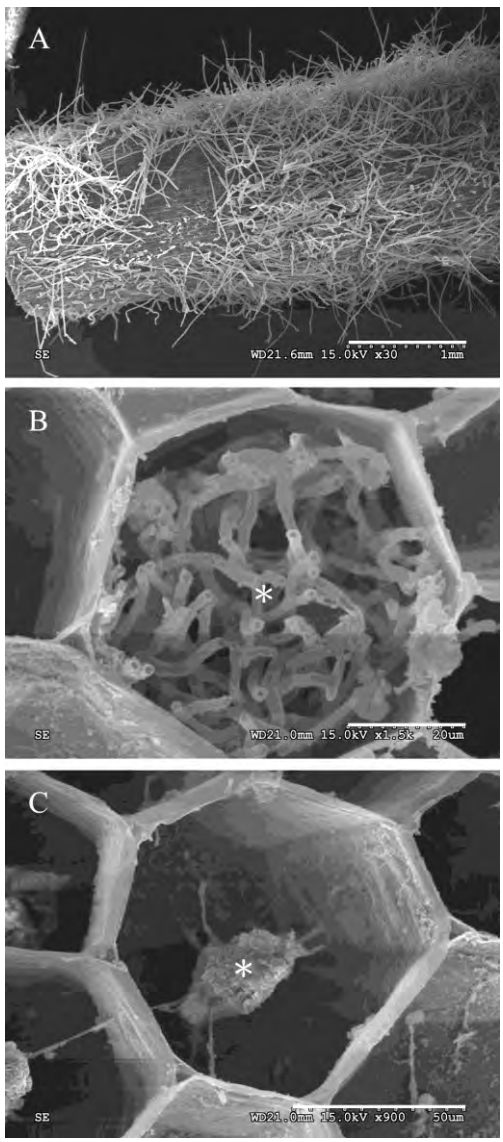

圖 2 阿里山根結蘭根菌結合體之微細構造。
A：表面有稠密的菌絲覆蓋；B：菌絲團構造(⁎)；C：老化菌絲團(⁎) 。

Fig. 2 Untrastructure of Calanthe arisanensis root-fungus association.
A: association surface with numerous fungal hyphae; B: young peloton; C: old peloton.

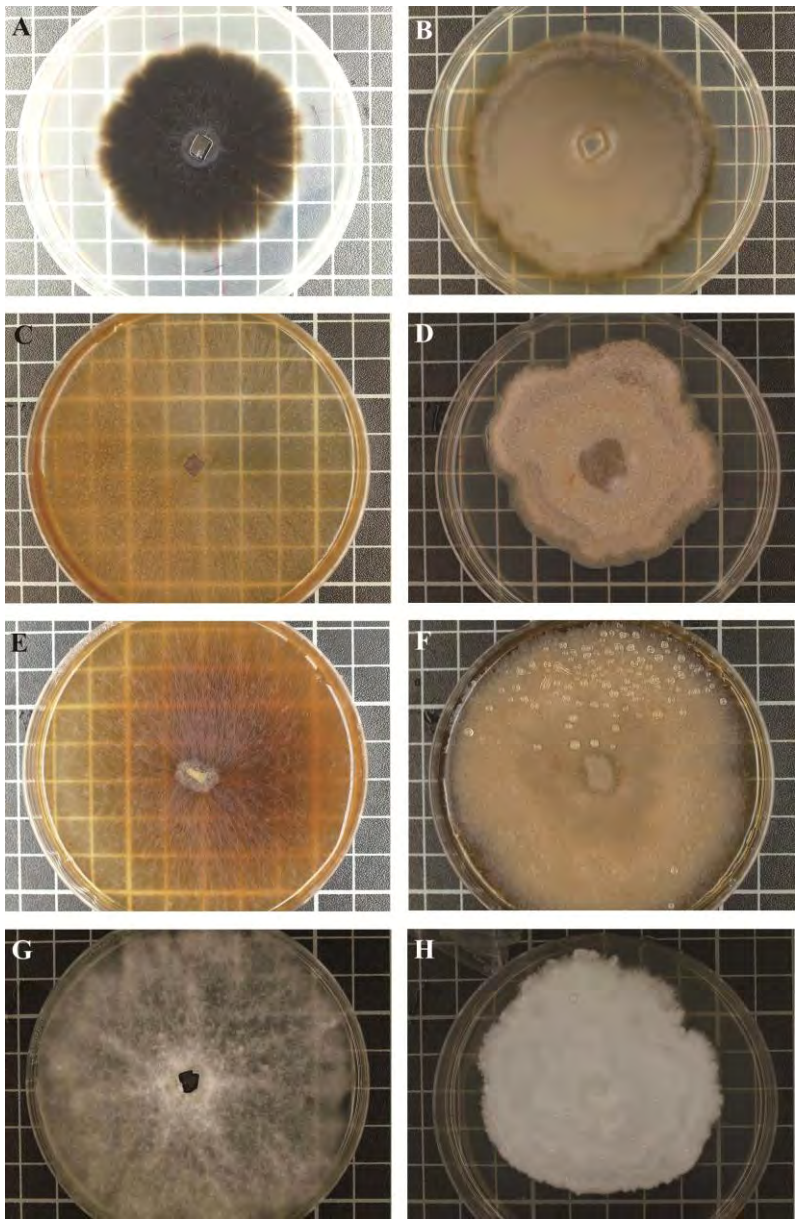

圖 3 阿里山根結蘭根系結合體內生菌之菌落形態。
A：Ca2 菌株 2% MEA 菌落形態；B：Ca2 菌株 PDA 菌落形態；C：Ca4 菌株 2% MEA 菌落形態；D：Ca4 菌株 PDA 菌落形態；E：Ca5 菌株 2% MEA 菌落形態；F：Ca5 菌株 PDA 菌落形態；G：Ca6 菌株 2% MEA 菌落形態；H：Ca6 菌株 PDA 菌落形態。

Fig. 3 Colony morphology of endophytes from *Calanthe arisanensis* fungal-root association after 27d. A and B, colony of Ca2 on 2% MEA and PDA, respectively; C and D, colony of Ca4 on 2% MEA and PDA, respectively; E and F, colony of Ca5 on 2% MEA and PDA, respectively; G and H, colony of Ca6 on 2% MEA and PDA, respectively.

表 1 阿里山根結蘭根菌結合體內生菌於不同培養基下之生長速率

Table 1 Growth rate of *Calanthe arisanensis* root-fungus association on and under different media and temperatures after 12ds

菌株 Strain	培養基 Medium	溫度 Temperature	平均值±標準偏差（mm/d） Means±SD
Ca2	MEA	23℃	2.6±0.1^{bc}
Ca2	PDA	23℃	2.4±0.1^{b}
Ca4	MEA	23℃	2.6±0.1^{c}
Ca4	PDA	23℃	3.1±0.1^{d}
Ca5	MEA	23℃	3.0±0.1^{d}
Ca5	PDA	23℃	3.1±0.1^{d}
Ca6	MEA	23℃	2.2±0.3^{a}
Ca6	PDA	23℃	3.1±0.1^{d}

All values reported as means±standard deviation for four replicates. (Duncan test, $P<0.05$)

Ca2菌株在2%MEA培養基中，菌落呈現深橄欖綠色（圖3A），生長速度爲2.6±0.1 mm/d；另在PDA培養基中，菌落呈現褐色（圖3B），生長速度爲2.4±0.1 mm/d。

Ca4菌株在2%MEA培養基中，菌落呈現淡褐色（圖3C），生長速度爲2.6±0.1 mm/d；另在PDA培養基中，菌落呈現淡咖啡色（圖3D），生長速度爲3.1±0.1 mm/d。

Ca5菌株在2%MEA培養基中，菌落呈現淡褐色（圖3E），生長速度爲3.0±0.1 mm/d；另在PDA培養基中，菌落呈現淡咖啡色（圖3F），生長速度爲3.1±0.1 mm/d。

Ca6菌株在2%MEA培養基中，菌落呈現灰白色（圖3G），生長速度爲2.2±0.3 mm/d；另在PDA培養基中，菌落呈現深白色（圖3H），生長速度爲3.1±0.1 mm/d。

IV、菌株分子生物分析

本研究利用ITS1-F/TW13爲引子組，萃取阿里山根結蘭根菌結合體中所分離之內生菌與親緣相近之菌株比對之結果如圖4所示：Ca2菌株與NCBI基因庫中糞殼菌綱菌株Sordariomycetes sp.（GQ153034）歸爲同群，支持度達89%；另Ca4與Ca5菌株經比對後和基因庫中*Neonectira* sp.（XJ243906）歸爲同群，支持度達100%；Ca6菌株與基因庫中炭角菌目菌株Xylariales sp.（HQ207035.1）及炭角菌屬菌株*Xylaria* sp.（EU678668）歸爲同群，支持度達100%。

鄭淑芬於2012年針對64種臺灣原生蘭科植物根部內生菌進行研究，共計分離出500多株內生眞菌，經形態及分子生物鑑定是以木黴菌（*Trichoderma* spp.）、鐮孢菌（*Fusarium* spp.）及炭角菌（*Xylaria* spp.）等三屬眞菌佔優勢。本次研究所分離純化之內生菌僅有Ca6菌株是應屬於炭角菌屬。

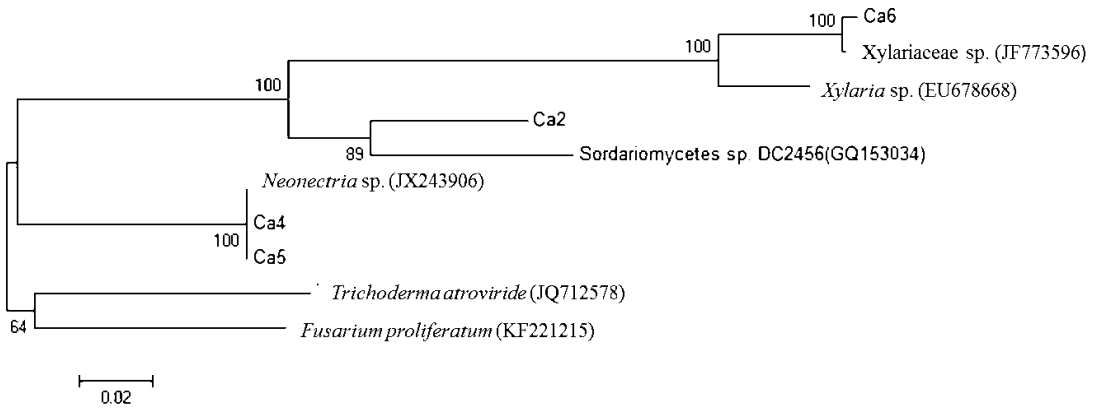

圖 4 阿里山根結蘭根系結合體之內生菌與基因庫中高相似度菌株之鄰接樹狀關係圖。

Fig. 4 Neighbor-joining phylogenetic tree based on rDNA ITS sequence data from endophytes isolated from *Calanthe arisanensis*, along with selected high similarity fungal species from GenBank.

結 論

本研究自南投縣郡大林道上所採集的阿里山根節蘭；在內生菌部分，於根菌結合體中共計誘導出 4 株內生菌，將這 4 株內生菌分別在 23℃及不同培養基（MEA 及 PDA）等處理下培育，其生長速度分別爲 Ca2 菌株（MEA: 2.6±0.1 mm/d 及 PDA: 2.4±0.1 mm/d）、Ca4 菌株（MEA: 2.6±0.1 mm/d 及 PDA: 3.1±0.1 mm/d）、Ca5 菌株（MEA: 3.0±0.1 mm/d 及 PDA: 3.1±0.1 mm/d）及 Ca6 菌株（MEA: 2.2±0.3 mm/d 及 PDA: 3.1±0.1 mm/d）；並經 ITS 分子序列比對後，內生菌 Ca2 菌株應屬於 Sordariomycetes、Ca4 和 Ca5 菌株應屬於 *Neonectria*、Ca6 菌株應屬於 *Xylaria*。在根菌結合體形態觀察部分，利用立體顯微鏡觀察可發現，有白色菌絲覆蓋在根菌結合體上；利用掃描式電子顯微鏡觀察，在根菌結合體橫切面之皮層細胞中發現菌絲團及老化的菌絲團等構造；以上特徵皆符合蘭花菌根之特性，因此可證實阿里山根節蘭根系結合體屬於蘭花菌根。

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Two Species of *Cololejeunea* (Family Lejeuneaceae) Newly Recorded in Taiwan

臺灣產疣鱗蘚屬(細鱗蘚科)2 個新紀錄種

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Abstract

Forty-eight species of the genus *Cololejeunea* (Lejeuneaceae) were reported from Taiwan. This paper describes and illustrates *C. platyneura* and *C. subkodamae* as two new distributional records in Taiwan, and provides information on their habitats and distribution.

摘　要

疣鱗蘚屬隸屬於細鱗蘚科，臺灣本屬原記載有 48 種。本文增加 2 個新紀錄種，假肋疣鱗蘚及擬兒玉氏疣鱗蘚(新擬中名)，文中附有此 2 種新紀錄種之形態描述、圖片及棲地與分布等資料。

Key words: *Cololejeunea*, *C. platyneura*, *C. subkodamae*, liverworts, Taiwan

關鍵詞：疣鱗蘚、假肋疣鱗蘚、擬兒玉氏疣鱗蘚、蘚類、臺灣

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Introduction

Cololejeunea is a large pantropical genus, represented in Taiwan by 48 species occurring from lowland to montane forest at ca. 2,500 m in elevation (Piippo 1990; Yang 2009, Wang *et al*. 2011, Ellis *et al*. 2013). It is characterized by (1) plants small to medium, yellowish green, pale green, whitish green to dull green, (2) stems usually zig-zag, branching of *Lejeunea*-type, (3) absence of underleaves, (4) leaf lobes spreading, ovate, obovate to narrowly lanceolate, insertion very short (only a few cells), apex rounded to acute-acuminate, margins entire to denticulate, often with seriate ocelli and/or hyaline marginal cells, (5) cells thin-walled, sometimes sigmoid, cuticle smooth or papillose-tuberculate, (6) oil-bodies hyaline or finely granular, (7) leaf lobules small or large, with a very narrow insertion to the stem, usually inflated and with a long keel, sometimes reduced or flattened with a short keel, apex with (0-)1-2 teeth, the hyaline papilla situated on the proximal base of the first tooth, but frequently displaced internally, or rarely on the tip of the first tooth, (8) perianths inflated 5-keeled or flattened 4- or 2-keeled, smooth or papillose, (9) vegetative reproduction by multicellular, disciform gemmae from the leaf surfaces (Gradstein *et al*. 2001; Yang 2009).

Our recent studies on genus *Cololejeunea* of Taiwan revealed two interesting taxa from Orchid Island. After consulting herbarium material and relevant literature (Herzog and Noguchi 1955; Mizutani 1961, 1966 & 1986; Tixier 1985; Zhu and So 1998; Zhu and So 2001; Yang 2009), they were identified as *C. platyneura* (Spruce) A. Evans and *C. subkodamae* Mizut., both new distributional records for the liverwort flora of Taiwan. We provide a brief description supplemented with illustrations and information on their habitats and local distribution.

Taxonomic Treatment

Cololejeunea platyneura (Spruce) A. Evans, Mem. Bot. Club. 8: 172. 1902.

Cololejeunea usambarica E. W. Jones, Trans. Brit. Bryol. Soc. 2: 434. 1954; nom. illeg.

Cololejeunea astyla Mizut., J. Hattori Bot. Lab. 29: 156. 1966.

假肋疣鱗蘚 Fig. 1

Description:

Plants small, brown to dark brown when dried. Stems up to 10 mm long, 60–80 μm in diameter, with leaves 0.78–0.98 mm wide, irregularly branched; ventral merophyte of the stem 1-cell wide; rhizoids numerous, in tufts. Leaves imbricate to contiguous, obliquely spreading; lobes oblong, 0.41–0.51 mm long, 0.31–0.37 mm wide; apex rounded or sometimes obtuse, flat to slightly incurved, margins subentire or denticulate caused by dorsal papillae. Cells of leaf lobes thin-walled; trigones distinct, intermediate thickenings sometimes present; marginal cells 21–26 × 12–19 μm, median cells 21–35 × 17–23 μm; and basal cells 38–45 × 16–24 μm. Cuticle papillose, except the basal part of leaf lobe and forming a vitta-like area. Leaf lobules oblong to ovate, 1/4–1/3 as long as the lobe, inflated; free lateral margin bordered with 4–6 elongated cells; apex truncate to slightly constricted, the first tooth unicellular and the second tooth indistinct, hyaline papilla usually at the proximal side of the first tooth, sometimes situated near the inner base of first tooth; keel slightly arched. Stylus absent. Androecia not seen. Gynoecia on a short lateral branch; female bracts smaller than stem leaves, the lobe oblong, 0.36–0.39 mm long, 0.1–0.12 mm wide, the lobule 2/3–6/7 the length of the lobe, oblong with an acute apex. Perianth pyriform, inflated, 4-keeled, immature. Asexual reproduction by discoid gemmae on the ventral surface of leaf lobe; mature gemmae not seen.

Habitat:

Cololejeunea platyneura is a pantropical epiphyllous species. It grows on leaves of vasicular plant in forest at 400–2,270 m in elevations (Mizutani 1966; Tixier 1985; Zhu and So 1998; Zhu and So 2001). Both specimens collected from Orchid Island were epiphytic, growing on tree barks and rotten wood, and often creeping on other bryophytes in the tropical monsoon forests at 300–552 m in elevations.

Distribution:

Africa, Brazil, China, Malaysia, and Vietnam (Mizutani 1966; Tixier 1985; Zhu & So 1998). New to Taiwan.

Specimens examined :

Taitung County: Orchid Island, Mt. Hongtou, on tree bark, mixed with *Lejeunea excils*, *Lopholejeunea subfusca*, *Radula acumminata*, *Frullania aoshimensis*, and *Taxithelium* sp., at ca. 300–552 m in elevations, 22°02' N, 121°31' E, 14 Aug. 1997, *Chih-Hsiung Hou BT-1940a* (**TAIE**, **TUNG**). Orchid Island, Mt. Hongtou, on rotten wood, mixed with *Lejeunea wightii*, *Lophlejeunea nigricans*, *L. eulopha*, *Metalejeunea cucullata*, *Radula* sp. and *Taxithelium* sp., at 300–552 m in elevations, 22°02' N, 121°31' E, 14 Aug. 1997, *Chih-Hsiung Hou BT-1999a* (**TAIE**, **TUNG**).

Remarks:

Cololejeunea platyneura belongs to subgenus *Cololejeunea* judged by the plants loosely appressed to the substratum, leaf cell

with dorsal papillae, inflated perianth with 4 keels, and the absence of distinct ocelli. It is readily distinguishable from all other congeners by (1) a vitta-like area in the basal part of leaf lobe, (2) stylus is absent, and (3) hyaline papilla is usually situated on the proximal side of the first tooth. *Cololejeunea platyneura* is a widely distributed pantropical species with diverse morphological variations. Taiwanese material is larger in both the plant body and cells size; otherwise, the diagnostic characters agree with the detailed descriptions and illustrations given by Mizutani (1966, as *C. astyla*), Tixer (1985, as *C. usambarica*) and Zhu and So (1998).

Cololejeunea subkodamae Mizut., J. Hattori Bot. Lab. 60: 448. 1986.

擬兒玉氏疣鱗蘚 Fig. 2

Description:

Plants minute, pale brown to yellowish brown when dried. Stems up to 3 mm long, 46–51 μm in diameter, with leaves 0.37–0.48 mm wide, irregularly branched. Leaves loosely imbricate to contiguous, obliquely spreading; lobes ovate, 0.20–0.26 mm long, 0.13–0.17 mm wide; apex obtuse or acute, slightly incurved, margin densely spinose. Cells of leaf lobes thin-walled; trigones indistinct and intermediate thickenings absent; marginal cells 7–10 × 5–8 μm, median cells 8–13 × 8–12 μm, basal cells 16–27 × 11–14 μm; dorsal protrusion spinose. Leaf lobules ovate, 1/2–2/3 as long as the lobe, inflated, ventral surface spinose, free lateral margin incurved, bordered with 3–4 elongated cells; apex 2-toothed, teeth crossing each other or approximate, first tooth 1–2 cells long, 1 cell wide, second tooth 1-celled, about 3 times as long as wide, a hyaline papilla on inner surface of the base of first tooth; keel arched, spinose. Sexual and asexual reproduction organs not seen.

Habitat:

Cololejeunea subkodamae usually grows on tree trunks, tree branches, tree bases, tree roots, rocks, boulders, decaying logs, and on leaves of ferns and seed plants in forest (Mizutani 1986). In China (Fujian, Guizhou, and Zhejian), it grows only from the leaves of herbs and small shrubs in moist forests at 400–1,250 m in elevations (Zhu and So 2001). In Taiwan, the specimens were collected from the fern fronds and climbers in the tropical monsoon forests of Orchid Island at 300–552 m in elevations.

Distribution:

China and Japan (Mizutani 1986; Zhu and So 2001). New to Taiwan.

Specimens examined :

Taitung County: Orchid Island, Mt. Hungtou, on climber, creeping on other bryophytes, mixed with *Lejeunea laii*, *L. wightii*, *L. excils*, *Cheilolejeunea ceylanica*, *Lopholejeunea subfusca*, *Radula* sp. and *Trichosteleum* sp., at 300–552 m in elevations, 22°02' N, 121°31' E, 14 Aug. 1997, *Chih-Hsiung Hou BT- 1957a* (**TAIE**, **TUNG**). Orchid Island, Mt. Hungtou, on leaves of fern [*Meringium denticulatum* (Sw.) Copel.] , mixed with *Cololejeunea geobelii* and *C. sigmoidea*, at 552 m in elevation, 22°03'10" N,

121°32' 5"E, 14 Aug. 1997, *Chih-Hsiung Hou BT-2325a* (**TAIE**, **TUNG**).

Remarks:

Cololejeunea subkodamae is characterized by (1) the spinose ventral surface of leaf lobule, (2) the obtuse or acute apex of leaf lobe, (3) the densely spinose margin of leaf lobe, and (4) the two teeth of leaf lobule usually crossing each other or approximate. It belongs to subgenus *Cololejeunea*, and may be confused with *C. spinosa* (Horik.) Pandé & Misra, but the latter differs in its smooth ventral surface of leaf lobule, and two teeth of leaf lobule never crossing each other. *Cololejeunea kodamae* Kamim. occurs in Yilan County, Taiwan (Ellis *et al*. 2013), also with spinose ventral surface of leaf lobule, and is closely related to *C. subkodame*. However, the lobular teeth are distant from each other, and the second tooth is as long as wide in *C. kodamae*. According to Mizutani (1986) and Zhu and So (2001), the finding of this species on Mt. Hongtou, Orchid Island, Taiwan (22°02' N, 121°31' E) represents an extension of its southern range of the distribution.

Key to the Species of *Cololejeunea* subg. *Cololejeunea* in Taiwan (Yang 2009, modified)

1. Leaf lobe with a vitta-like area in the basal part; stylus absent……..….... *C. platyneura*
1. Leaf lobe without a vitta-like area in the basal part; stylus usually present……..…..…….2
2. Dorsal protrusion of leaf cells spinose ……..3
2. Dorsal protrusion of leaf cells shortly cylindrical or semispherical …..…….....….....6
3. Leaf lobule spinose on ventral surface ……4
3. Leaf lobule smooth on ventral surface ..……5
4. First and second teeth of leaf lobule distant from each other, second tooth short, about as long as wide…….…….*C. kodamae*
4. First and second teeth of leaf lobule crossing each other or approximate, second long, about 3 times as long as wide ….....*C. subkodamae*
5. First and second teeth of leaf lobule distant from each other; vitta like ocelli weakly developed .....…… *C. spinosa*
5. First and second teeth of leaf lobule usually crossing each other or approximate; ocelli absent …..…….. *C. haskarliana*
6. Leaves usually falcate; hyaline papilla at the proximal side of the base of first tooth…..... ..…..... *C. tenella*
6. Leaves not or slightly falcate; hyaline papilla on the inner surface of the base of first tooth...7
7. First tooth of leaf-lobule acute……...…8
7. First tooth of leaf-lobule hammer-shaped or rounded……..……...….9
8. Leaf lobes with an acute to obtuse apex, base of dorsal margin nearly entire; vitta like ocelli absent …….....*C. shikokiana*
8. Leaf lobes with a rounded to rounded-obtuse apex, base of dorsal margin often spinose-dentate; vitta like ocelli usually weakly developed..…….... *C. schmidtii*
9. Leaf lobe obovate, apex sometimes with 1-5 minute teeth; perianth nearly smooth ……..… ..... *C. dozyana*
9. Leaf lobe broadly ovate, apex without teeth; perianth strongly papillose …..….*C. macounii*

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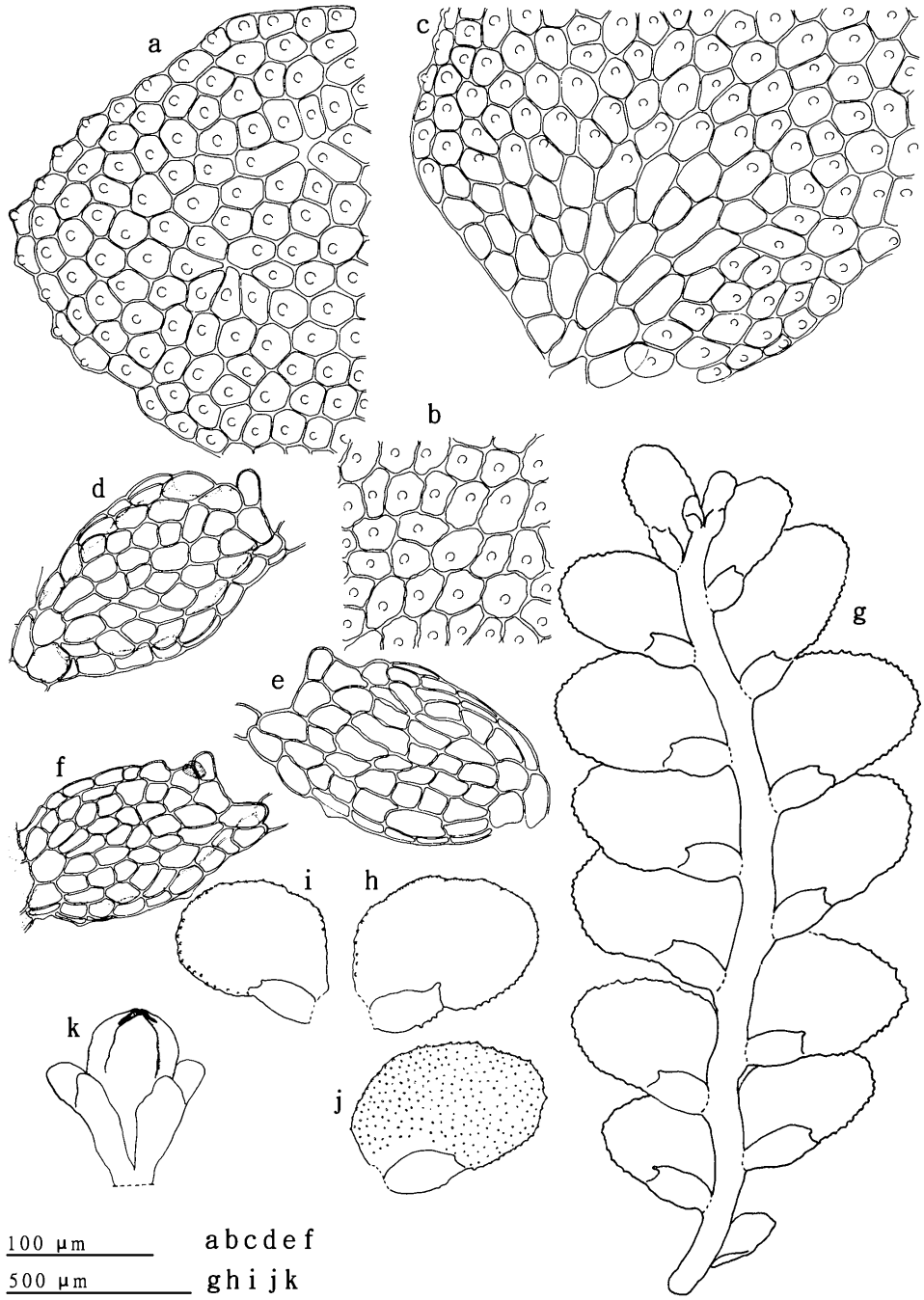

Fig. 1. ***Cololejeunea platyneura*** (Spruce) A. Evans a. Cells of leaf apex. b. Median cells of leaf lobe. c. Basal cells of leaf lobe. d–f. Leaf lobules. g. Portion of the plant, ventral view. h–j. Leaves. k. Gynoecium, ventral view, immature. (All drawn from *Chih-Hsiung Hou BT-1940a*).

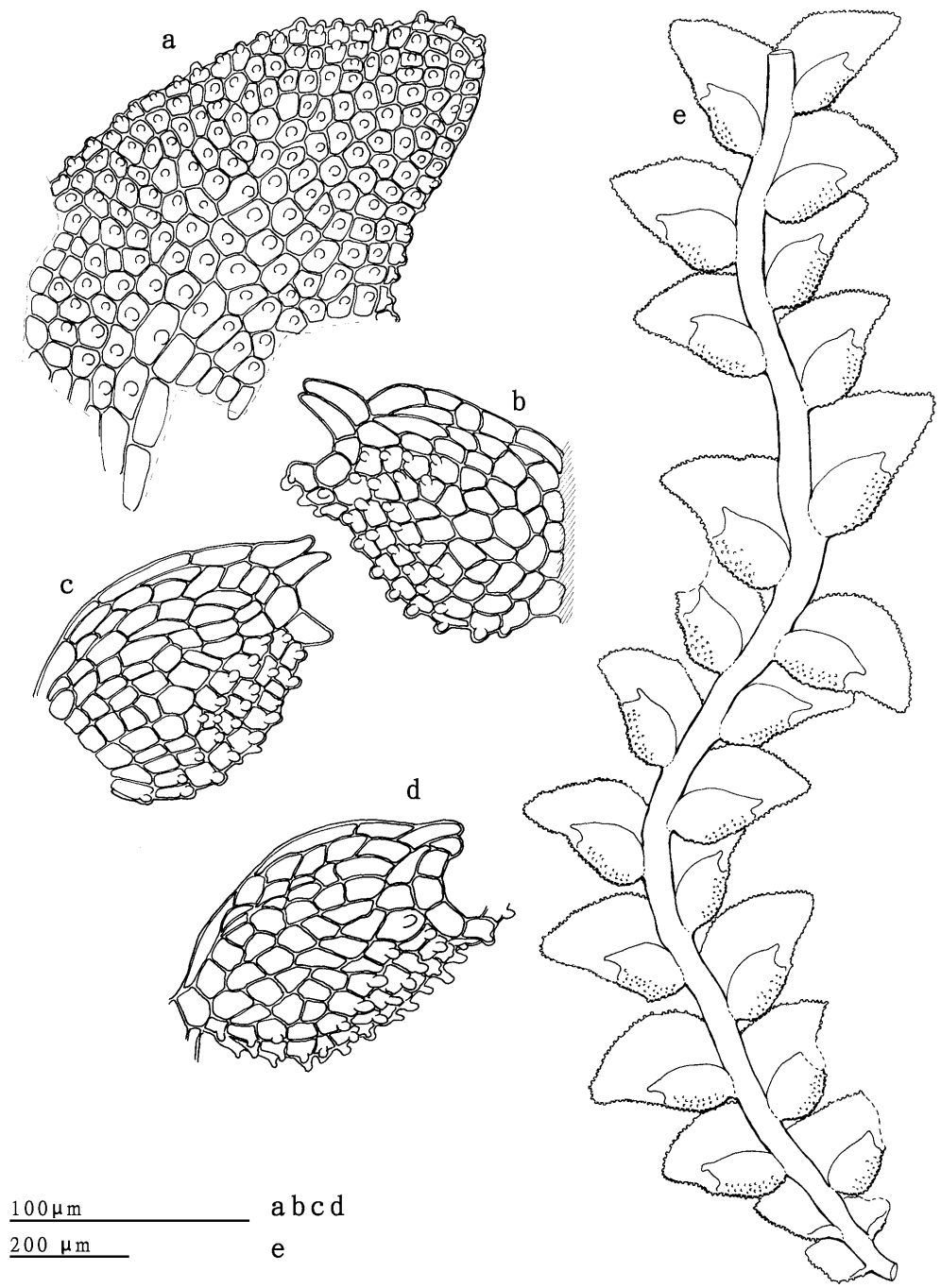

Fig. 2. ***Cololejeunea subkodamae*** Mizut. a. Cells of leaf lobe, dorsal view. b–d. Leaf lobules. e.Ventral view of a portion of sterile plant. (All drawn from *Chih-Hsiung Hou BT-1957a*).