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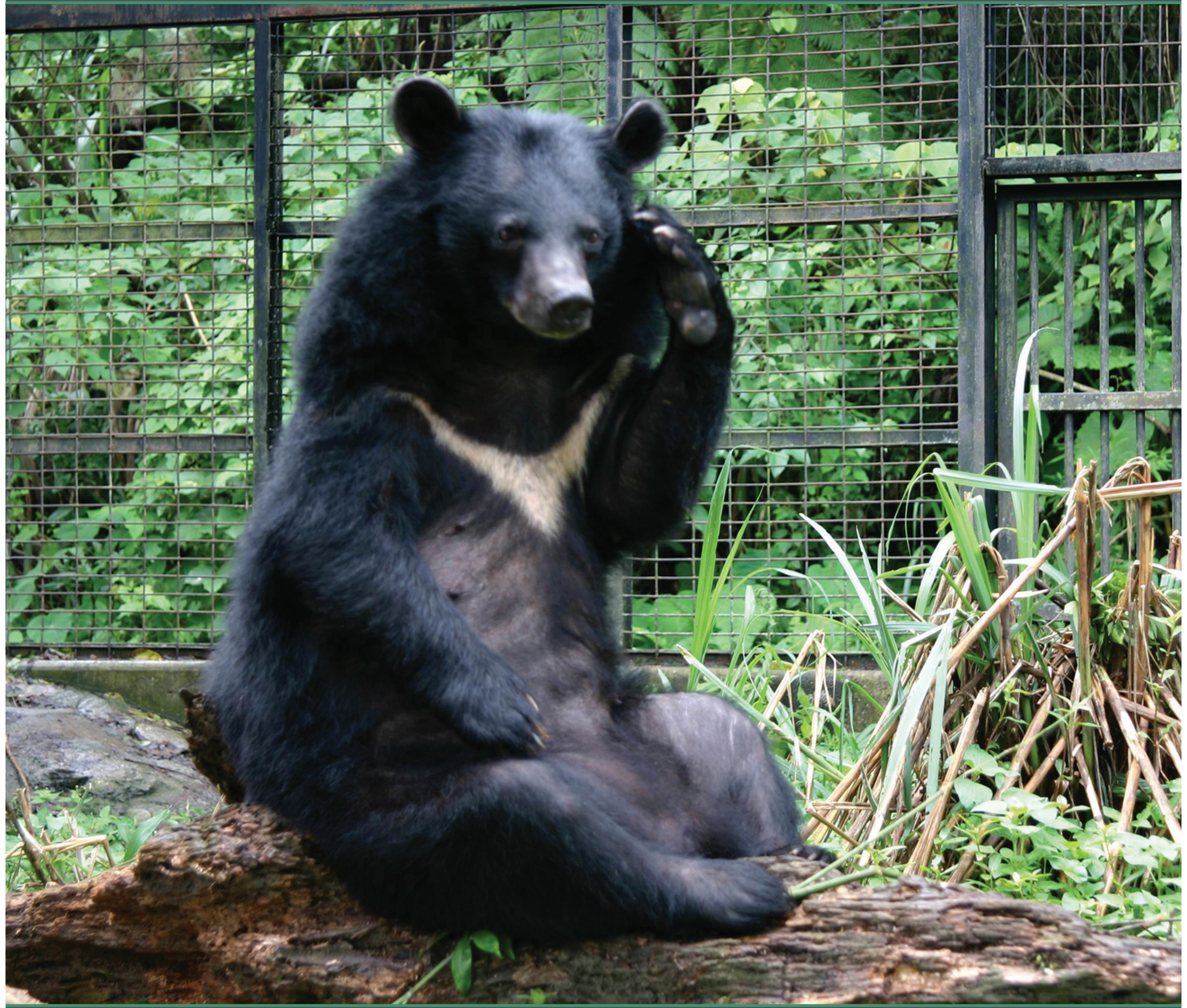

封面圖說／
台灣黑熊(*Ursus thibetanus formosanus*)各種行為所代表的意義，我們所知有限。(楊吉宗　攝)

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Contents

《台灣生物多樣性研究》通告

《台灣生物多樣性研究》源自於《特有生物研究》，自1999年創刊以來，已出版11卷，期間承蒙各界研究學者關注與支持，特此申謝。

聯合國宣示2010年是國際生物多樣性年，除了希望凸顯生物多樣性保育的重要，也期待透過全球合作，讓人類找出維護生物多樣性的方法，值此時刻，《特有生物研究》更名爲《台灣生物多樣性研究》，提供國內外學者專家發表有關生物多樣性或自然保育之研究報告，以促進學術交流，並落實本土保育工作，並自2010年第12卷第1期起增加發行期數，即由每年1月、7月的每半年一期，改爲每年1月、4月、7月及10月出刊的一年四期季刊，特此通告周知，誠摯邀請各界先進同道不吝賜稿，讓本刊成爲生物多樣性研究與交流的最佳園地。

Announcement

“Endemic Species Research” has been published semi-annually since January 1999. We are thankful to all contributors who in the past selected this journal as the final home for their manuscripts. In correspondence to the United Nations’ declaration of 2010 as “International Year of Biodiversity”, starting January 2010, “Endemic Species Research” will be replaced by “Taiwan Journal of Biodiversity”. This new journal will be published quarterly in the months of January, April, July and October. It will continue to serve as a permanent home for manuscripts resulted from hard work of many scholars of various disciplines in the field of biodiversity and natural conservation. “Taiwan journal of Biodiversity” cordially invites and welcomes scholars around the world, particularly those in Taiwan, for submission of their manuscripts, making this journal to be one of the best forums in the field of biodiversity.

圈養台灣黑熊攝食與繁殖的關係探討

An Exploration on Relationship between Food Intakes and Reproductive Success of Captive Formosan Black Bears (*Ursus thibetanus formosanus*)

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摘　要

圈養的台灣黑熊(*Ursus thibetanus formosanus*)雌熊於交配且受孕後，分別在懷孕初期及懷孕中期增加給飼量及其營養濃度，結果在懷孕中期增加給飼時正常生產，但在懷孕初期增加給飼時有發生早產現象，本研究認爲懷孕初期增加給飼量增加了早產的機率，其原因是促使孕酮(progesterone)的分泌濃度提前上升，且其最高峰的水準未達一定的程度，可能因此改變了母體子宮環境暨其子宮腔液的蛋白質組成，使著床後的胚胎發育未與子宮環境同步而引起。野外熊隻於懷孕中期後獲得較多食物，是長期配合食物季節性變化的一種韻律性適應，而熊隻演化出的延遲著床(delayed implantation)是一種內在生理適應。因此，若對圈養雌熊於懷孕初期增加飼糧的給飼量，可能擾亂牠們性類固醇荷爾蒙的自然分泌與延遲著床機制相關的韻律性，此可能是促使異常生殖的主因。

Abstract

After the captive female Formosan black bear (*Ursus thibetanus formosanus*) mated and was fertilized, her daily food ration was increased from either early-pregnancy or mid-pregnancy. The results showed that the female bear had normal deliveries when received the food increment from the mid-pregnancy but had preterm deliveries when received the food increment from the early-pregnancy. The food increment from the early-pregnancy might result in higher probability of the preterm delivery which was because the rise of progesterone secretion ahead of time, and it didn't reach to some certain higher level. Hence, it probably led to change the uterine environment and the protein composition of the uterine luminal secretion, of which became unsuitable for the fetus development after embryo implantation. In nature there is a seasonal change in food composition and abundance, and wild female bears consume food with nutritional values at the stage of mid-pregnancy than that at the stage of early pregnancy. It is an adaptive synchronization of the internal physiological rhyme of the bears with the embryo delayed implantation to the seasonal rhyme of food abundance in the natural environments. Accordingly, we hypothesize that increasing the feeding of captive bears at the early pregnancy may disturb their natural sexual steroid hormone secretion rhyme associated with the delayed implantation mechanism, which was probably the main reason to cause abnormal reproduction.

關鍵詞：台灣黑熊、攝食量、早產、延遲著床

Key words: *Ursus thibetanus formosanus*, diet intake, preterm delivery, delayed implantation

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緒言

圈養的台灣黑熊多在春、夏季交配，受精後之胚胎經約 5 個月的延遲著床(delayed implantation)後再著床發育，之後再經 2 個多月的發育後於冬季生產，總懷孕期約 7.5 個月(楊等 2003)，此與美洲黑熊(*Ursus americanus*)的情況類似(Foresman and Daniel 1983; Seager and Demorest 1986; Flowerdew 1987; Domico and Newman 1988; Hunt and León 1995)。亞洲黑熊(*U. thibetanus*)的懷孕狀況亦類似，約在 5-8 月間交配，懷孕期約 5.5-8 個月，於當年的 12 月至隔年的 3 月間生產(高等 1987; 楊等 1991; 苟 1991; 孔等 1998)，日本棕熊(*U. arctos uesoensis*)也有類似情形(Tsubota *et al*. 1991)。現生 12 科食肉目中有 7 科有延遲著床現象，

熊科是其中之一，其演化的假說有多種，主要是與食物、育幼或爲適應環境以求物種的存續(mead 1989)。延遲著床的主要步驟及調控機制如圖 1，受精卵分裂形成囊胚(blastocyst)後即暫停發育，在子宮內浮游約 5 個月再著床，其調控因素包括日照、內分泌及營養等而影響子宮內的環境(Renfree and Calaby 1981; Mead 1989)。其中營養的部分，所涉及的包括攝食食物的品質、量及其季節性的變化，圈養的情況下雖然沒有季節性變化，但有飼糧成分、給飼量與給飼方式的不同。本文主要目的在探討不同給飼策略對台灣黑熊繁殖產生的影響，並探討其可能的原因。

材料與方法

試驗雌熊有 2 隻，爲圈養於行政院農業委員會特有生物研究保育中心低海拔試驗站，其中雌熊 1 號(2001 年時約 7 歲)，來自民間捐贈，2001-2005 年間均與同爲民間捐贈之 1 隻雄熊(2001 年時約 11 歲)配對，開始配對在 2-3 月之間，配對的方式是把飼養在隔壁鄰舍之雌、雄熊籠舍中間的柵門打開，讓其自由進出及互動，俟有交配行爲後，至無互動行爲爲止(通常在有交配行爲後約 2 週就無互動行爲)，再予以隔離分欄飼養；雌熊 2 號(2000 年時 4 歲)來自雪山地區，被盜獵，右後腳踝以下截肢，2000 年及 2007 年分別與來自壽山動物園(年齡不詳)及來自花蓮玉里(約 4 歲)的雄熊配對，操作之情況如雌熊 1 號。

給飼的飼糧以蘋果、柳丁、紅蘿蔔、地瓜、木瓜、玉米、麵包、雞蛋、豬肉、肉骨及成犬飼料等不同組成調配，分成 A 及 B 兩種飼糧。A 飼糧之乾基含粗蛋白 11.5%，熱能 4.15 Kcal/g；B 飼糧含粗蛋白 17.0%，熱能 4.38 Kcal/g，後者含粗蛋白較高，即單位飼糧之營養濃度較高，兩者鈣磷比同爲 1.8:1.0 (表 1)。平時給飼量以 A 飼糧之乾物量 1.0-1.1 kg/100 kg 體重計算，以一週 7 天的量縮爲 6 天於早上給飼(週日不給飼，且豬肉及肉骨全集中在週六給之)；增加給飼是在交配後以 B 飼糧給飼，給飼的時間與方式和 A 飼糧相同。雌熊 1 號增加給飼量的時機分別爲：一、2002 及 2003 年在懷孕初期(數天)即增加給飼量；二、在 2001、2004 及 2005 年在懷孕中期(2.5-4 個月)才增加給飼量(如圖 1 之 A)，增加給飼的方式爲每隔 7 天或 14 天增加給飼量 10%或 20%。雌熊 2 號於 2000 及 2007 年分別於疑似交配後的懷孕初期及中期增加給飼量，增加給飼的方式爲每隔 7 天增加 10%。2 隻雌熊增加給飼均視當時之食慾及攝食狀況而定，若增加給飼量後可連續 2-3 天以上把全部飼糧吃完，再依比例予以增加，若沒完全吃完就不再增加。在懷孕後期攝食量漸減少時，給飼量維持先前的量不減少，每日記錄給飼量及攝食量。

糞孕酮(fecal progesterone)的檢測，每週以非侵入法(non-invasion)採取雌熊之糞便 2-3 次，收集後先置於-20°C，處理時取糞材 1g 加入 3 ml 分析緩衝液，震盪混合 10 min，100°C 加熱 10 min，再震盪混合 20 min，以 3,500 rpm 離心 30 min 後抽取上清液，再以酵素免疫分析法(enzyme immunoassay, EIA)分析雌二醇(estradiol)及孕酮(progesterone)濃度，使用之抗體及酵素免疫結合體取自中興大學獸醫學系生理實驗室，標準液爲購得(Sigma，編號 P9776)，微滴盤酵素免疫分析儀是「anthos 2001」(Austria 製)。

結果與討論

試驗熊隻的給飼量，平時飼養以蛋白質需求量估約 11%之乾物基含量並依體重給予，以限制過食並防過於肥胖。懷孕初期因考慮懷孕時營養需求可能增加，故飼糧改爲含蛋白質 17%，量亦予以增加。懷孕中期才增加給飼，是因懷孕初期的食慾增加，從外表上看並不十分

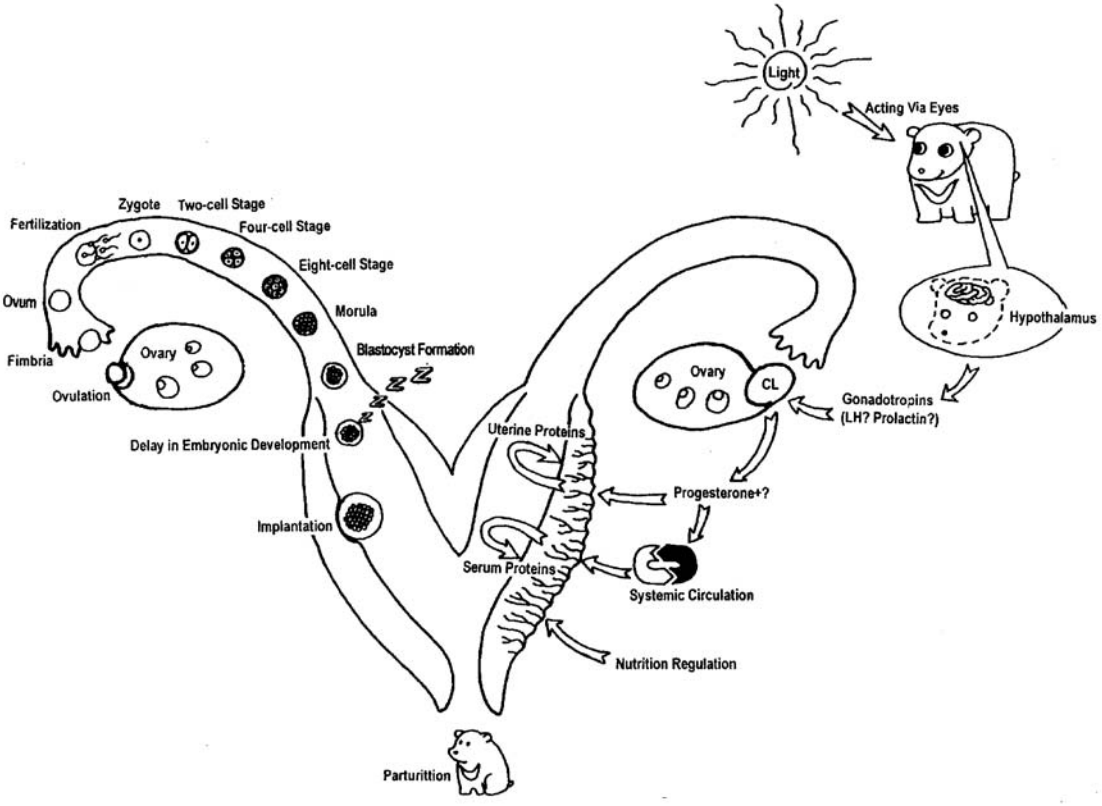

圖 1. 黑熊胚胎延遲著床的主要步驟及調節機制概圖(仿自 McDonald 1980; Renfree and Calaby 1981; Mead 1989)。

Fig. 1. Major steps and regulatory mechanisms of embryo delayed implantation in black bear (after McDonald 1980; Renfree and Calaby 1981; Mead 1989).

表 1. 本試驗用之 2 種飼糧的乾物基營養組成分

Table 1. The nutrient contents (dry matter basis) of diets A and B used in the study

Diets	C. Protein (%)	C. Fat (%)	C. Fiber (%)	Calcium (%)	Phosphorus (%)	Energy (Kcal/g)
A	11.5	5.2	6.3	1.8	1.0	4.15
B	17.0	7.6	7.2	1.8	1.0	4.38

明顯，故於中期才增加給飼。這種交配後懷孕時的不同增加給飼方式，是依現場實際飼養狀況而調整，因並未有台灣黑熊懷孕時飼養管理的相關資訊可資應用。

攝食的情況，平時之日糧以乾物量 1.0-1.1 kg/100 kg 體重給飼。交配之後，雌熊 1 號逐漸增加給飼的百分比如表 2，以 2002 年最多，達 160%，2001、2003、2004 及 2005 年爲 60%-70%。懷孕初期給飼並攝食之總增加量達 160%者是在每 14 天增加 20%之下，同在懷孕

初期而每 7 天增加 10%者，最後總增加量爲70%；懷孕中期增加給飼者爲每 7 天增加 10%，最後均爲增加 60%。懷孕初期或中期增加的百分比與最後總增加量似無關連，影響最後總增加量的原因並不清楚。攝食量於產前 35-75 天迅速降低，並於產前 7-30 天完全拒食(如表 2 及圖 2 之 B)。雌熊 2 號給飼及攝食的情況如圖 3，2000 年在交配後疑似懷孕初期即增加給飼，最後增加達 50%，攝食量在 87 天之前把給飼量全部吃完，之後逐漸下降，但並未完全拒食；2007 年在疑似懷孕的中期才增加給飼量，總增加量達 70%，攝食量在懷孕 160 天之前全部吃完，之後亦逐漸下降。依攝食的情況，發覺懷孕初期或中期增加給飼的方式對攝食量降低及拒食的時間似無關連，但對懷孕初期增加給飼之雌熊並未繁殖成功，其間似有著相關性。

雌熊 1 號在懷孕初期即增加給飼量的 2002 及 2003 年發生早產，胎兒出生不久即死亡。懷孕中期之後增加給飼量的 2001 及 2005 年均順利生產並育成仔熊，2004 年發生死產。雌熊 2 號是在交配後增加給飼量，不管在懷孕初期或中期，其攝食量亦伴同給飼量增加而增加，但該隻雌熊後來並未生產，因與雄熊的交配行爲從錄影檔難以辨識清楚，故只稱其疑似交配，最後呈現假懷孕現象，其現象包括食慾增加、後期攝食量漸減、多蜷縮在窩巢等，在其後期仍維持少量的進食(如圖 3)，而與雌熊 1 號於分娩前完全拒食(如圖 2 之 B)有所差別。

表 2. 雌熊 1 號之給飼、攝食紀錄及繁殖狀況表

Table 2. The feeding procedure, daily food intakes, and reproduction of the female bear #1 in 2001-2005

Items	2001	2002	2003	2004	2005
Time of changing diet formulas and increasing daily food ration	Mid-pregnancy	Early-pregnancy	Early-pregnancy	Mid-pregnancy	Mid-pregnancy
Intervals of increasing daily food ration (days)	7	14	7	7	7
% in total increased food ration	60	160	70	60	60
Refusing food intake before delivery of cub (days)	9	26	17	30	7
Total gestation (days)	226	179	169	253	225
Cub status	Survival	Preterm delivery (died 2 days after birth)	Preterm delivery (died half days after birth)	Stillbirth	Survival

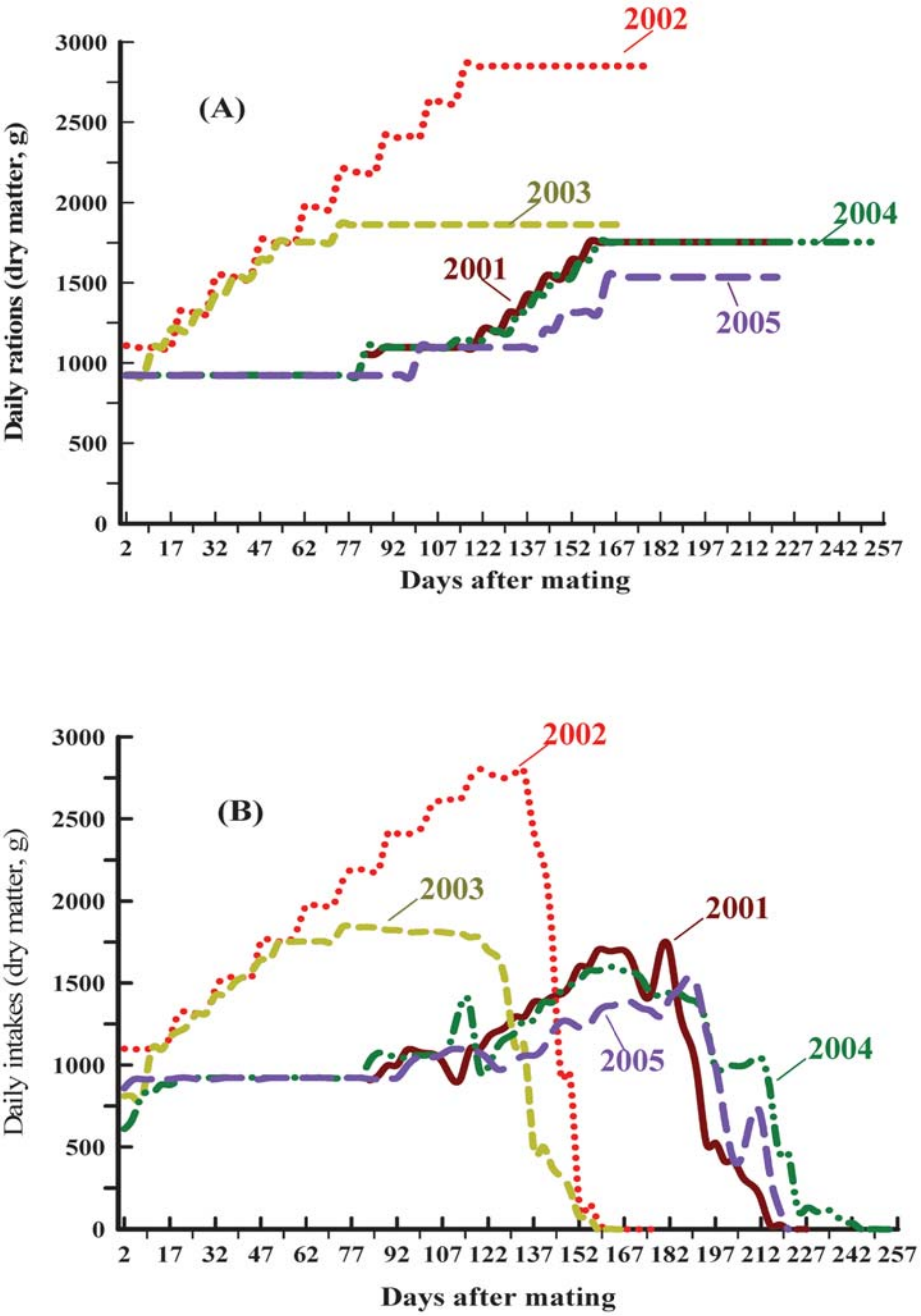

圖 2. 雌熊 1 號於 2001-2005 年懷孕期間給飼(A)及攝食(B)情形，其中 2001 及 2005 正常生產，2002 及 2003 早產，2004 死產。

Fig. 2. Daily rations (A) and intakes (B) of bear female #1 during the periods of pregnancy in 2001-2005, resulting in normal deliveries in 2001 and 2005, preterm deliveries in 2002 and 2003, and stillbirth in 2004.

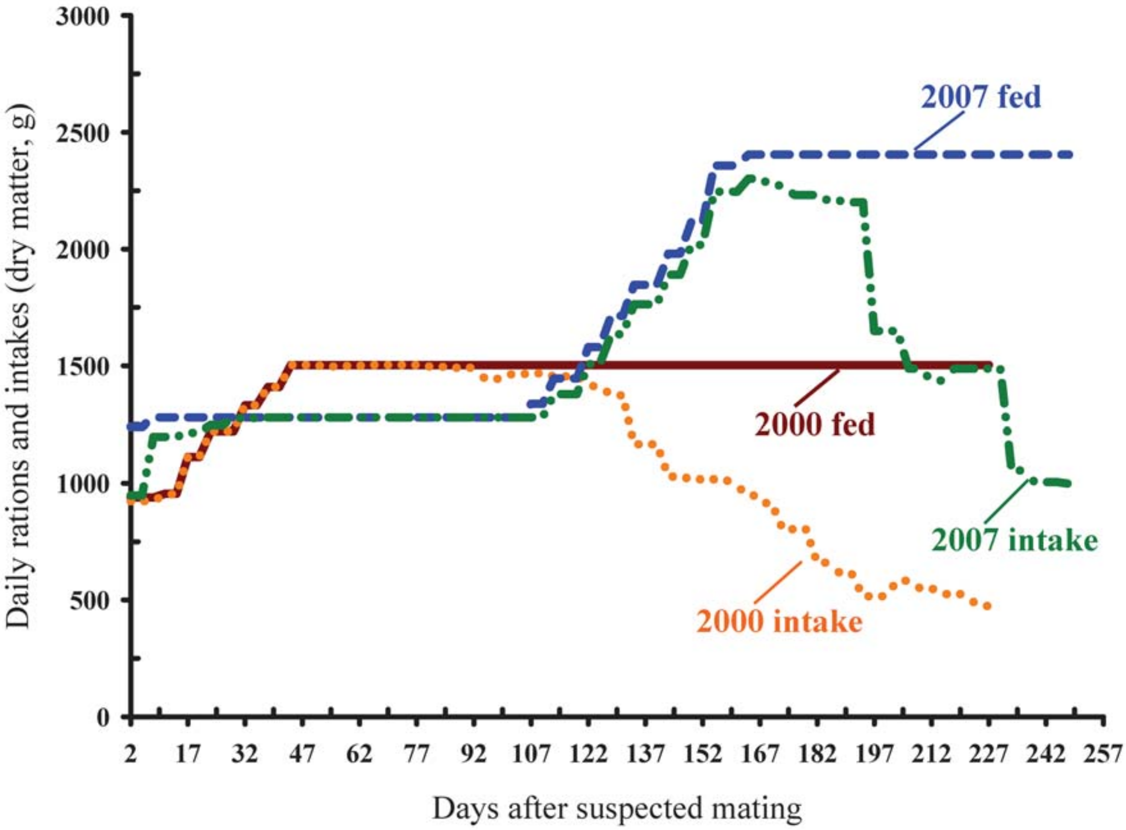

圖 3. 雌熊 2 號於疑似交配後之日糧與攝食情形，增加給飼的時間 2000 年在疑似懷孕的初期，2007 年是在中期，二年均呈現假懷孕且食慾增加。

Fig. 3. Daily rations and intakes of the female bear #2 after suspected mating with the ration increments at early-pregnancy in 2000 and at mid-pregnancy in 2007, both resulting in pseudopregnancy with increasing appetite.

植物性食物的營養成分有季節性的變化，粗蛋白含量隨其生長及新生之合成代謝的進行而增加(Greenwood and Barnes 1978)，Scheirs *et al*. (2002)報告食葉昆蟲所攝食的葉子，除陸續長出新芽可維持營養水準之外，成熟及老化者品質將降低。反芻動物放牧以豆科爲主之牧草之粗蛋白含量，亦有季節性的變動(Well and Cooper 2001; Söter *et al*. 2007)。豆科植物所含粗蛋白是隨成熟階段而異，最高時可達 15-25%，低時約 14-15%，熱帶地區禾本科牧草(grass)含粗蛋白約 5-10%，季節性差異顯著(Luttleton 1973)。麋鹿(*Alces alces*)選食的植物種類及食用的部位在夏天含粗蛋白量有增或減(Kubota *et al*. 1970)，白尾鹿(*Odocoileus virginianus* ssp.)啃食的草、枝、葉及嫩芽含粗蛋白則爲春天較高，夏天急速降低，冬天維持在低濃度(Smith *et al*. 1956)。Sfougaris *et al*. (1996)稱克里特野山羊(*Capra aegafrus cretica*)攝食的植物類群，含粗蛋白以禾本科牧草在夏天最低，豆科(legume)及非草屬草本植物(forb)則春天高，灌木(shrubby)則春、夏漸低，秋、冬逐漸增高。Robbins (1993)綜合一些報告，

稱生長初期的禾本科植物、草本植物、嫩枝、葉或芽，其乾物基含蛋白質高達 20-30%，但隨著成熟逐漸降至 3-4%。

熊隻攝食植物的季節性變化，Hellgren *et al.* (1988)曾敘述美洲黑熊(*U. americanus*)春天攝食多汁及草本的植物，夏天多攝食軟的果實，秋初爲大量殼斗科(Fagaceae)的果實，秋末及冬天則吃常綠性灌木及藤蔓類的果實。Lander *et al.* (1979)亦有報告美洲黑熊攝食季節性食物的營養，冬季入洞休眠前含較高的無氮抽出物(nitrogen-free extractive)及脂肪，出洞後的食物含較高的蛋白質。中國四川的亞洲黑熊(*U. thibetanus*)季節性攝食的主要植物，春天及夏初爲非草屬的草本植物及灌木，再轉換爲灌木的漿果(如薔薇科的懸鈎屬*Rubus* spp.、虎耳草科茶藨子屬 *Ribes* 及樟科新木薑子屬 *Neolotsea*)，夏中及夏末則爲樹木果實(如薔薇科樫屬*Prunus*)，秋天爲殼斗科堅果類(如櫟屬 *Quetcus*) (Reid *et al*. 1991)。果實(或漿果)之乾物基含粗蛋白量亦變異很大，在 2.0-16.3%，其生長以 6-7 月較多(Sakai and Carpenter 1990)。台灣黑熊爲雜食性動物，春、夏季主要以綠色植物爲主食，秋冬則以果實爲主並捕食較多的偶蹄類動物，季節性所攝食的植物春天以嫩葉芽、夏季以樟科(Lauraceae)及薔薇(Rosaceae)的果實、秋冬則以殼斗科的堅果爲主(王及黃 1999; 王及陳 1991; Hwang *et al.* 2002)，殼斗科青剛櫟(*Cyclobalanopsis glauca*)的果實約於 10 月開始成熟，其爲台灣黑熊重要的食物來源(黃等 2009)，唯所攝食食物的營養組成分資料尚付之闕如。動物性食物的營養成分，哺乳類、鳥類及魚類之乾物基粗蛋白爲 22-91%，無脊椎動物則在 30-88%，差異範圍很大(Robbins 1992)。台灣黑熊所攝食的動物性食物包括哺乳類、鳥類、魚類，昆蟲爲常見覓食的種類，植物性食物從根、莖、葉、花到果實都吃，且有偏好喜好吃果實的現象(作者等，未發表)。綜合上述，由於台灣黑熊在野外攝食的食物種類多而雜，攝食類別的比例變動不定，難以確知，就以季節性食物的蛋白質濃度而言，推測亦隨季節性變化而變化，所攝食的營養在春、夏較低，而入秋後較高。

台灣黑熊多在春、夏季交配，若在野外，交配後懷孕初期的季節性食物的粗蛋白濃度不高，進入秋、冬季之後的懷孕中期及後期，攝食植物的果實大多數在此季節逐漸成熟而有較高的蛋白質，特別是脂肪，且於此季節大量攝食並儲存脂肪，以備休眠或待產。熊隻的季節性繁殖是適應全球氣候變遷長期演化而來(Spady *et al.* 2007)，胚胎發育的過程與食物季節性的變化成了一種韻律性。簡言之，也就是發情交配受孕之後約 5 個月的胚胎延遲著床期間，值春、夏季的營養呈現在較低濃度狀態；而胚胎著床之後 2 個多月的發育期，值入秋之後，有較多機會獲得營養成分較高的果實或捕食較多的動物性食物，可供過冬或懷孕的營養需求。

雌熊 1 號 2001-2004 年間交配後孕酮的變化情形如圖 4，圖中是以 3 個樣本測值繪成移動平均趨勢線，其有中斷部分是熊隻沒攝食致無排遺可資分析。座標 X 軸起始點爲交配日期，分別在 3-5 月不等。2001 年交配後約 5 個月孕酮才逐漸上升，產前最高峰達 408.7 ng/g，懷孕 225 天後正常生產；2002 及 2003 年發生早產，其孕酮分別在交配後未達 5 個月即顯示上升，2 年的最高峰之値分別爲 135.7 ng/g 及 101.9 ng/g，懷孕期分別爲 179 天及 168 天後均發生早產；2004 年於交配後約 7 個月孕酮才上升，最高峰爲 148.4 ng/g，懷孕期共 253 天(比正常多 28 天)，最後發生死產。2005 年於 3 月底交配，11 月 12 日生產，懷孕期 225 天，順利產下雙胞胎。懷孕期動物異常生產(早產或流產、死產)的原因包括近親繁殖、胚胎發育畸形、懷孕次數多、不當增重、緊迫及營養不當等多種(Gravett 1984; 龔 1992; Hutchins *et al.* 1996; Schieve *et al.* 1999; Schieve *et al.* 2000)，本文所探討生產狀態的熊隻爲

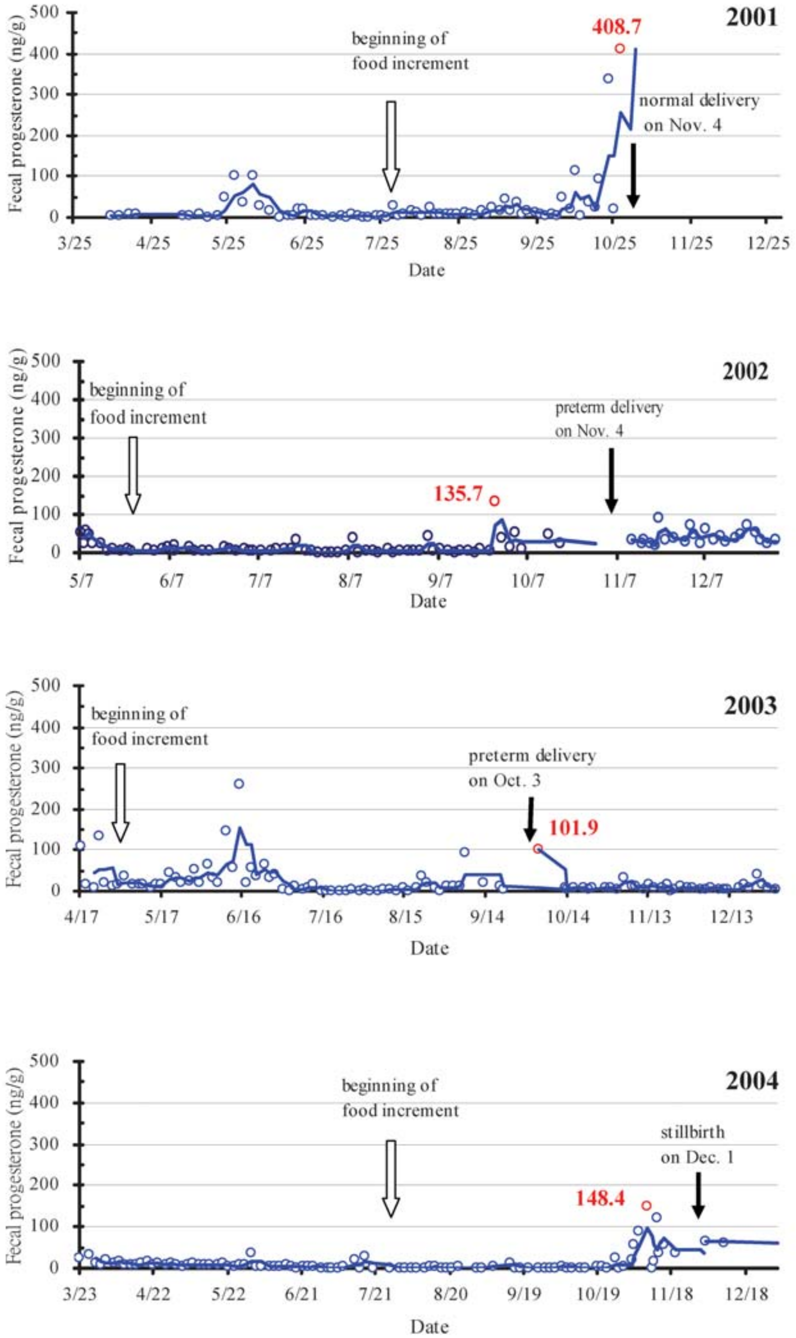

圖 4. 雌熊 1 號於 2001-2004 年交配後之孕酮每 3 個樣品移動平均趨勢線、增加給飼時間及分娩狀況(空心箭頭爲開始增加日糧，實心箭頭爲生產時間及狀況)。

Fig. 4. Trends 3-measurements moving average of fecal progesterone concentrations of the female bear #1 after mating in 2001-2004 (open vertical arrows, the time of beginning daily ration increments; solid vertical arrows, time and status of cub deliveries).

同一個體，可能影響其生產的環境因素均相似，最主要差別在於交配後增加給飼的時間不同。2001及2004年的正常生產及發生死產之增加給飼是在懷孕中期，而2002及2003年發生早產的是在懷孕初期。懷孕初期增加給飼可能影響孕酮分泌，使其於交配後未達5個月就已達最高峰，或雖未達最高峰但已顯見上升，可能因此促使胚胎提早著床。雌熊2號因沒有生產且2007年未分析孕酮的變化，故其孕酮與胎兒發育的關係就無從敘述。孕酮的上升與胚胎的著床是一致的(palmer *et al.* 1988; Sato *et al.* 2000)，且著床後會比基礎值增2-3倍(Foresman and Daniel 1983)。雌熊1號在2002及2003年產前約2個月孕酮的分泌最高值比2001年少約3倍，可能因無足夠濃度而無法使胎兒在正常環境下發育。2004年增加給飼是在懷孕中期，但其孕酮濃度亦僅爲2001年的1/3，其發生死產的原因可能與孕酮的濃度較低有關，但是否與前2年連續懷孕且早產有關，不得而知，通常熊隻是2年1胎。因此，綜觀其情，孕酮濃度較低並非全然是懷孕初期增加給飼的關係，但懷孕初期增加給飼使孕酮濃度較低似有較高的機率。

營養之攝食與孕酮及胎兒的關係，女豬(gilt)在懷孕初期給予高水準營養的飼料會經由增加胰島素(insulin)的分泌而促使孕酮增加，孕酮是影響胚胎存活的媒介(Jindal *et al.* 1977; Beltranena *et al.* 1991; Jindal *et al.* 1996)。女豬在懷孕初期給予高營養水準而增加胚胎死亡，可能是經由孕酮分泌而與子宮液內之蛋白質(如retinol binding protein)有關，因而使特定懷孕階段之子宮與胚胎的變化未同期化(Soede *et al.* 1999)。豬的子宮液內的蛋白質量會隨孕酮增加而增加，其變化會影響胚胎的發育與存活(Knight *et al.* 1973; Chew *et al.* 1982; Vallet *et al.* 1998)。Garrett *et al.* (1988)亦發現肉牛在懷孕初期，孕酮會調控胚胎的發育與生長。孕酮使胎兒異常生長是藉由增進或降低胚胎的發育而起，其扮演著因與果的雙重關係角色，可能是透過血液傳送進入子宮腔，或直接刺激子宮內膜釋出生長因子(如insulin-like growth factor-Ⅰ and Ⅱ)而改變子宮內的環境(Geisert *et al.* 1991; Barnes 2000; Velazquez *et al.* 2008)。人與靈長類亦有報告胚胎著床會受孕酮的影響(Sengupta and Ghosh 2000)，人與畜牧的胚胎與子宮環境間的同步(synchrony)是正常懷孕生產不可或缺的(Barmes 2000)。日本黑熊(*U. thibetanus japonicus*)繁殖週期孕酮濃度與子宮內膜細胞增生厚度及子宮腺體的面積呈正相關(Yamane *et al.* 2009)，Hellgren *et al.* (1989)亦報告認爲黑熊的代謝產物(如血液內的總蛋白、球蛋白等)與食物品質的季節性變化有緊密關係且具有其韻律(rhythm)，韻律的變化包括體重、食物消耗及繁殖等，內源性循環的律動，關係到動物的生理與行爲，進而至植物的季節性變化。因此，本研究推測懷孕初期給予較高濃度的營養及較多的飼糧，可能有較多的機率改變熊隻對食物季節性變化的適應，擾亂懷孕初期約5個月的胚胎延遲著床，以及產前約2個月孕酮濃度未升高到達相當程度，而影響子宮內膜細胞的增生及其腺體的發育，進而影響子宮內的環境。因此，改變了長期演化下來於懷孕中期以後才能獲較高營養水準時機的內在適應，致使子宮環境暨其子宮液組成未能與胎兒發育同步，而影響了正常繁殖。

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領角鴞和黑冠麻鷺繁殖期與氣溫的相關性

Relationships between Air Temperature and Reproduction Timing for Collard Scops Owl (*Otus bakkamoena*) and Malayan Night Heron (*Gorsachius melanolophus*)

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摘　要

在全球暖化日益受到重視的當下，國際間已有許多研究探討氣候變遷對野生動物繁殖的影響，但台灣針對此議題的野外研究仍十分缺乏。本研究利用特有生物研究保育中心野生動物急救站的落巢幼鳥案例，分析鳥類繁殖時間和氣溫的相關性，以案例數最多的領角鴞(*Otus bakkamoena*)和黑冠麻鷺(*Gorsachius melanolophus*)爲研究對象。本研究先利用圈養的幼鳥建立研究對象的生長曲線，再選取中部地區的案例，以送達急救站時的落巢幼鳥體重回推該巢的繁殖時間，總計獲得領角鴞 9 年 133 巢和黑冠麻鷺 5 年 56 巢的繁殖資料。將各年繁殖起始時間與繁殖季前各個月份的平均氣溫進行迴歸分析，領角鴞和黑冠麻鷺的繁殖時間分別與前一年 11 月($p < 0.05$)和 12 月($p < 0.01$)的月均溫有顯著相關，該月份氣溫上升則隔年的繁殖時間將會提前。在當前暖化的趨勢下，野生動物繁殖期提前對生態的影響值得關注。

Abstract

As global warming has drawn our increasing concerns, many studies have focused on whether climate change would affect the timing of wildlife reproduction. We used orphaned nestling data of two avian species, the Collard scops owl (*Otus bakkamoena*) and the Malayan night heron (*Gorsachius melanolophus*), obtained by the Wildlife First Aid Station of the Endemic Species Research Institute of Taiwan to analyze the relationships between air temperatures and their reproduction timing. A growth curve model was built and daily growth rate by weight of each of the species was determined with the data of captive nestlings at the station. Egg deposition date of each of the orphaned nestlings was then estimated with the growth model. There were 133 orphaned nestlings of the Collard scops owl from Central Taiwan for nine years and 56 nestlings of the Malayan night heron for five years. A regression analysis was conducted between the estimated egg deposition dates and the monthly average air temperatures prior to the breeding seasons. The egg deposition dates were significantly correlated with the average air temperatures of the months of previous November ($p < 0.05$) for the Collard scops owl, and previous December ($p < 0.01$) for the Malayan night heron. When monthly average air temperatures of these months rose, the egg deposition dates of the two species proceeded earlier in the following year. Further studies are needed to examine whether other avian species have the same trend of air temperature and breeding time relationship and what is the mechanism.

關鍵詞：全球暖化、氣溫、繁殖時間、落巢幼鳥、生長曲線

Key words: global warming, air temperature, breeding time, orphaned nestling, growth curve

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緒言

聯合國跨政府氣候變遷小組(Intergovernmental Panel on Climate Change, IPCC)在 2007 年的報告中提出警告，過去 100 年來(1905-2005)全球地表平均溫度已增加 0.74°C，到本世紀末時有可能再增加 1.8-4°C (IPCC 2007)。全球暖化導致氣候改變已是不爭的事實，對生態系也產生嚴重的衝擊，對象涵蓋所有分類群的生物，從個別物種的族群量和生活史、生物群落的物種組成，到生態系的結構與功能皆受到影響，對生物多樣性保育而言更是一大威脅 (McCarty 2001; Parmesan and Yohe 2003)。

相較於其他類群的野生動物，鳥類較接近人類的生活環境且容易觀察，受民眾關注的程度高，因此非常適合作爲環境變遷的指標物

種。國際間已有許多關於氣候變遷影響鳥類的研究，包括食物供給失衡(Barbraud and Weimerskirch 2001)、分布範圍改變(Thomas and Lennon 1999; Hiych and Leberg 2007)、遷徙和繁殖時序錯亂(Crick 2004)等。例如極區、高海拔和受限於特定棲地的物種，若無法改變其分布範圍以因應氣候變化，就會面臨棲地減少以及更多的物種競爭，成爲氣候變遷下的首批犧牲者(Parmesan 2006)。

研究顯示受暖化影響，某些候鳥在秋季離開繁殖地的時間會延後，春季北返的時間則會提前(Gilyazov and Sparks 2002; Wilson 2007)，例如歐洲雨燕(*Apus apus*)在春季返回義大利的時間與 4 月份平均溫度有關，溫度每升高 1°C 則抵達時間提早 3.8 天，1982-2006 年間已平均提早約 6 天(Rubolini *et al*. 2007)。繁殖方面，北美洲樹燕(*Tachycineta bicolor*)共 3,450 個巢的紀錄中，發現受到春季溫度變暖的影響，自 1959-1991 年間燕子的平均繁殖時間提早了 9 天(Dunn and Winkler 1999)；在英國對 36 種鳥類 57 年的研究中，有 37%產卵時間的變化趨勢明顯受到氣候變遷的影響，研究者並預測到 2080 年時，75%的種類產卵時間都會提前(Crick and Sparks 1999)。

鳥類繁殖時間會受到溫度影響，可能來自生理限制和食物資源兩個因素。溫度被認爲是決定鳥類地理分布中最重要的因素，就是因爲鳥類的生理限制，各自有適合生存的溫度範圍(Root 1988; Wiens 1989; Mock 1998)，而產卵和之後的繁殖行爲是非常耗能的過程，冬、春季溫度提早回升則可提前解除能量限制，因而提早繁殖(Stevenson and Bryant 2000)。鳥類育雛期是否能跟食物資源的高峰期重疊，是影響繁殖成功率的重要關鍵，而溫度提早回升也可能導致食物資源的高峰期提前，因此鳥類可能會調整繁殖時間以因應食物的物候週期變化，但若提前繁殖的程度與食物物候改變的程度不一致，就會導致食物供給失衡(Visser *et al*. 2003)。

鳥類生態受全球暖化影響嚴重，Crick (2004)認爲應對更多物種在不同的地區進行全面且深入的研究。世界上各地區受溫室效應影響的程度不盡相同，台灣位於暖化程度較明顯的東亞季風區，百年來增加的溫度約是全球平均的兩倍(劉等 2008; 陳 2008)，然而相關氣候變遷對生態系影響的研究卻十分有限，不但難以掌握生態系受衝擊的層面與程度，更無法訂定適宜的因應策略(李等 2005)。目前台灣已有研究者利用電腦模擬探討全球暖化如何影響鳥類分布(廖 1996; 李等 2005)以及應用於保護區規劃(王 2008)，然而遷徙和繁殖方面需要長期的調查監測，相關研究成果仍付之闕如。

特有生物研究保育中心野生動物急救站(以下簡稱急救站)於 1993 年 12 月成立，截至 2008 年底累計病例數 4,233 件，其中 82.6%是鳥類。分析 2006-2007 年間 714 筆鳥類病例資料，數量最多的依序爲領角鴞(*Otus bakkamoena*) (13%)、黑冠麻鷺(*Gorsachius melanolophus*) (11.6%)和鳳頭蒼鷹(*Accipiter trivirgatus*) (7.6%)，傷病原因則以創傷(41.5%)和落巢幼鳥(35.7%)爲主(林等 2008b)。本研究嘗試以落巢案例數最多的領角鴞和黑冠麻鷺爲研究對象，利用落巢案例的頻度推估其野外的繁殖週期，並分析其繁殖時間和氣溫的相關性，期能作爲台灣野生動物受氣候變遷影響之參考。

材料與方法

本研究以領角鴞和黑冠麻鷺爲研究對象，領角鴞屬於鴞形目(Order Strigiformes)鴟鴞科(Family Strigidae)的小型夜行性猛禽，是第二級的保育類，體長 19-25 cm，廣泛分布於中低海拔森林以及靠近山區的鄉村地帶，於樹洞中繁殖，每巢卵數 4.0±0.8 個，孵化時間 27.8±1.8 天(林 2003a)。黑冠麻鷺屬於鸛形目(Order Ciconiiformes)鷺科(Family Ardeidae)，體長約

47 cm，於低海拔森林底層活動，過去被認爲是稀有留鳥，但近年來普遍出現於都市內的校園和公園綠地。黑冠麻鷺築巢於喬木上，每巢卵數3.4±0.8個，孵化時間30.4±1.2天(姚 2002)。

本研究需要利用幼鳥體重判斷其日齡，因此必須先獲得領角鴞和黑冠麻鷺幼鳥日齡與體重關係的生長曲線。領角鴞資料來自於急救站內收容的個體，分別在2008和2009年春季經配對後進行繁殖的3窩領角鴞，共計8隻幼鳥。照養人員於每日下午提供1次食物，食物以預先安樂死之無特定病原(specific pathogen free, SPF)小鼠(約26-28g重)和1日齡小雞(約35-38g重)爲主。分量計算方式爲成鳥1隻小鼠，幼鳥則視成長狀況給半隻至1隻小鼠，同一窩領角鴞的食物(小鼠或等重小雞)一次給予後，由成鳥自行進食並餵養幼鳥，依此份量隔日通常還會發現殘餘食物，幼鳥體重每3-6天記錄1次。黑冠麻鷺資料來自於2007和2008年分別有2隻和3隻約僅1-2日齡的落巢幼鳥，在人工飼養下的體重變化資料，食物以SPF小鼠和朱文錦爲主，將其切碎塞食至幼鳥肚略鼓脹，初期1日5次(每次約7-10g)，中後期則遞減爲3次(每次約25-30g)。

整理急救站自1993年成立至2008年底的案例資料，挑選出領角鴞和黑冠麻鷺落巢幼鳥的案例，由於這些案例大多來自中部縣市(台中縣市、南投縣、彰化縣、雲林縣和嘉義縣)，因此將其他縣市的零星案例予以排除。部分案例是來自颱風或大規模施工等事件造成的大量落巢，或是獸醫初診發現有消瘦或脫水的症狀時，同樣均不予採計。剩餘案例則依送達急救站時的體重參考生長曲線回推其日齡，領角鴞自20日齡(約150g)、黑冠麻鷺自30日齡(約500g)起體重變化趨緩，回推日齡時易出現較大誤差，此時須再參考部分案例上註明的換羽狀況，以進行日齡上的修正，難以判斷的案例則不納入分析。得知落巢幼鳥的日齡後，再依林(2003a)和姚(2002)所調查野外卵孵化所需天數，來估計該隻幼鳥在巢中被產下卵的時間。若有兩隻以上的幼鳥來自同一個巢，以體重最輕的個體被產下卵的時間作爲該巢開始繁殖的時間。

由於急救站每年所接獲的第一筆落巢幼鳥案例不可能精準的反映出野外第一個巢的繁殖時間，因此本研究取領角鴞案例中每年前1/3繁殖的巢平均的產卵時間(例如某年有15個巢，則取前5個巢的平均產卵時間，以每年1月1日起的天數計算，若除不盡則取至小數點第2位)，做爲該年繁殖季起始的基準時間。黑冠麻鷺的樣本數較少，因此取前1/2的巢數。有研究指出中部地區的領角鴞，在都會區的繁殖時間早於原始棲地(林 2003b)，其都會區和原始棲地的定義分別爲1,000人/km^2以上和100人/km^2以下(林等 2008a)，爲檢驗上述的繁殖季起始時間是否受到案例來源地不同所影響，本研究以案例來源的鄉鎮爲單位，計算每年來自都會區和原始棲地的案例比例，並比較比例與繁殖季起始時間有無顯著趨勢，人口數據取自內政部2008年底的統計資料。

溫度資料取自中央氣象局位於中部平地至低海拔的4個氣象測站(台中、梧棲、日月潭和嘉義)月均溫的平均值。將繁殖季起始時間與繁殖季前各個月份的平均大氣溫度進行迴歸分析，以求得兩者間的關係。

結果

一、生長曲線

急救站收容的兩對領角鴞在2008-2009年間所繁殖出的3窩共8隻幼鳥，離巢所需天數爲25.3±0.5天，前30日齡平均每隻測量6.0±2.6次體重，測量間隔3-9天不等，總計獲得47筆體重資料，自22日齡(約150g)起體重增加的情況會趨緩，體重(y)與日齡(x)的關係式爲：$y = 0.0028x^3 - 0.335x^2 + 13.043x - 12.454$ (R^2 =

0.978) (圖 1)。黑冠麻鷺幼鳥離巢所需天數參考姚(2002)爲 36.0±2.1 天，急救站 2007-2008 年共 5 隻人工飼養的黑冠麻鷺幼鳥，前 40 日齡平均每隻測量 12.8±4.4 次體重，測量間隔 1-7 天不等，總計獲得 64 筆體重資料，自 30 日齡(約 500g)起體重也會趨緩，體重與日齡的關係式爲：$y = -0.015x^3 + 0.5571x^2 + 13.248x + 7.682$ ($R^2 = 0.989$) (圖 2)。

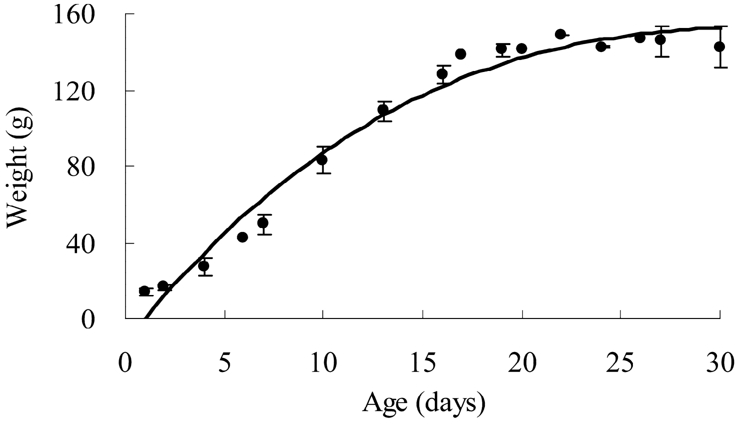

圖 1. 2008-2009 年在野生動物急救站所繁殖的 8 隻領角鴞幼鳥體重與日齡關係的生長曲線(圖爲平均重量 ± SD)。

Fig. 1. The growth curve of 8 captive Collard scops owls at the Wildlife First Aid Station, 2008-2009 (solid circles, average weights; horizontal bars, standard deviations).

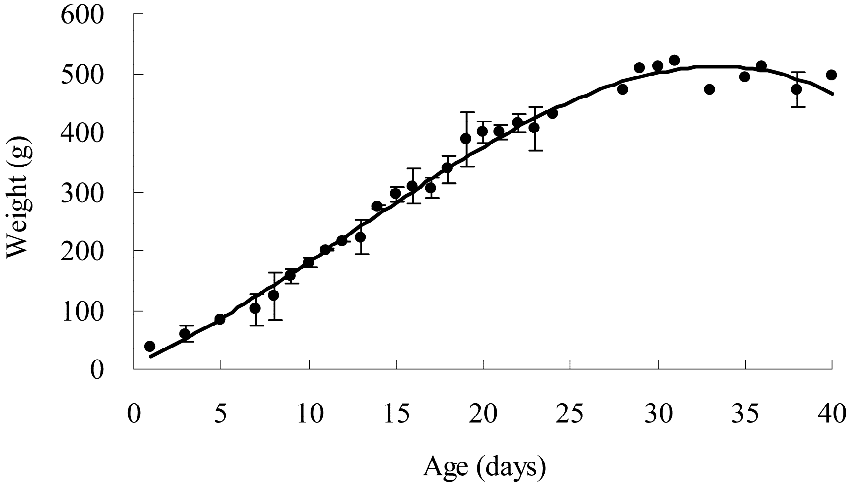

圖 2. 2007-2008 年野生動物急救站人工飼養的 5 隻黑冠麻鷺幼鳥體重與日齡關係的生長曲線(圖爲平均重量 ± SD)。

Fig. 2. The growth curve of 5 captive Malayan night herons at the Wildlife First Aid Station, 2007-2008 (solid circles, average weights; horizontal bars, standard deviations).

二、案例整理與來源地分析

急救站於 1993-2008 年共接獲落巢幼鳥1,074 隻，數量最多的領角鴞和黑冠麻鷺分別有 216 隻和 113 隻，排除中部地區以外的零星案例，也排除沒有體重紀錄的案例後(1998 年以前大多沒有體重紀錄)，兩種鳥可用的案例分別是 175 隻和 85 隻，來自 133 和 56 個巢(表1)，先利用生長曲線回推日齡，再參考林(2003a)和姚(2002)所調查野外卵孵化所需天數，可獲得各個巢開始繁殖的時間(表 2，表 3)。領角鴞除了 1999 年之外，2000 年到 2008 年間每年都有 10 個巢以上的資料，因此計算繁殖起始時間時排除了 1999 年，共有 9 年的資料；黑冠麻鷺樣本數較少，僅採用 2001、2002、2006-2008 等 5 個年份，每年的巢數在 7 巢以上。本研究計算出各年度領角鴞和黑冠麻鷺的繁殖起始時間如表 4。

領角鴞和黑冠麻鷺案例來源地屬於都會區(包括台中市、豐原市、大里市、大雅鄉、烏日鄉、大肚鄉、太平市、彰化市、社頭鄉、南投市、斗六市、嘉義市)的分別有 37 巢和 10 巢，來自原始區(包括和平鄉、信義鄉)的僅有 3 巢和 2 巢(表 4)，將領角鴞和黑冠麻鷺各年度的繁殖起始時間和都會區案例的比例進行迴歸分析，結果均無顯著趨勢($p > 0.5$)。

三、繁殖時間與氣溫分析

將領角鴞與黑冠麻鷺每年繁殖起始時間與繁殖前各個月份平均大氣溫度進行迴歸分析(表 5)，結果跟 11 月均溫的顯著值最高($p <$

表 1. 本研究所採用的領角鴞和黑冠麻鷺落巢案例來源縣市統計

Table 1. Sources of orphaned nestlings of the Collard scops owl and the Malayan night heron

Sources (county)	Collard scops owl	Malayan night heron
Taichung	15	6
Nantou	98	28
Changhua	7	9
Yunlin	1	5
Chiayi	12	8
Total	133	56

表 2. 以落巢案例回推領角鴞產卵時間，共 133 個巢在不同年份和月份的數量分布。爲表示同一個繁殖季，將 12 月排在隔年的 1 月之前

Table 2. Egg deposition dates estimated for 133 orphaned nestlings of the Collard scops owl

Month\Year	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	Total
December					2		1	1	1		5
January	2	1	1		1						5
February		1	2	2	3	1	1	1	1	2	14
March	1	6	8	11	5	9	6	5	4	20	75
April		1	3	1		1	3	3	5	5	22
May		1	1		1	2	1		2		8
June					2	1					3
July				1							1
Total	3	10	15	15	14	14	12	10	13	27	133

表 3. 以落巢案例回推黑冠麻鷺產卵時間，共 56 個巢在不同年份和月份的數量分布

Table 3. Egg deposition dates estimated for 56 orphaned nestlings of the Malayan night heron

Month\Year	2000	2001	2002	2003	2004	2005	2006	2007	2008	Total
March								2		2
April	1	3	2	1	1	2	3	2	2	17
May	1	1	2		2			4	6	16
June		1	1			1	6	3	1	13
July		2					1	1		4
August			2				1	1		4
Total	2	7	7	1	3	3	11	13	9	56

表 4. 2000-2008 年領角鴞和黑冠麻鷺繁殖起始時間以及各年度來自都會區和原始棲地案例的比例

Table 4. Egg deposition dates of the Collard scops owl and Malayan night heron estimated from the orphaned nestlings obtained from urban and rural areas, 2000-2008

Year	Collard scops owl				Malayan night heron			
	Egg deposition date (1=1 Jan)	Nests distribution (%)			Egg deposition date (1=1 Jan)	Nests distribution (%)		
		Urban	Transitional area	Rural		Urban	Transitional area	Rural
2000	42.6	20	80	-				
2001	48	20	73.3	6.7	110.7	28.6	57.2	14.2
2002	59	26.7	73.3	-	122.9	33.3	66.7	-
2003	42	28.6	71.4	-				
2004	31.9	28.6	64.3	7.1				
2005	59	25	75	-				
2006	36.6	20	80	-	137.1	18.2	81.8	-
2007	43	15.4	76.9	7.7	112.6	15.4	76.9	7.7
2008	56	29.6	70.4	-	113.1	33.3	66.7	-
Total		28.5	69.2	2.3		21.3	74.5	4.2

表 5. 將領角鴞與黑冠麻鷺每年繁殖起始時間與繁殖前各個月份平均氣溫進行迴歸分析結果

Table 5. Statistics of the regressive analyses between estimated egg deposition dates of the Collard scops owl and Malayan night heron and the monthly average air temperatures prior to the breeding seasons

Month	Collard scops owl (n=9)		Malayan night heron (n=5)	
	R^2	*p-value*	R^2	*p-value*
October	0.219	0.203	0.094	0.615
November	0.505	0.032	0.016	0.841
December	0.311	0.119	0.942	0.006
January	0.015	0.752	0.012	0.861
February	0.156	0.293	0.026	0.797

0.05)，關係式如下：

$$D = -7.66T + 207.63 \ (df = 8)$$

D 爲自 1 月 1 日起的天數，T 爲 11 月均溫，即溫度每升高 1 度，繁殖季前 1/3 巢的平均繁殖時間會提早 7.3 天(圖 3)。黑冠麻鷺繁殖起始時間則與 12 月均溫達到顯著性($p < 0.01$)，關係式如下：

$$D = -9.73T + 292.01 \ (df = 4)$$

即溫度每升高 1 度，繁殖季前 1/2 巢的平均繁殖時間會提早 9.7 天(圖 4)。

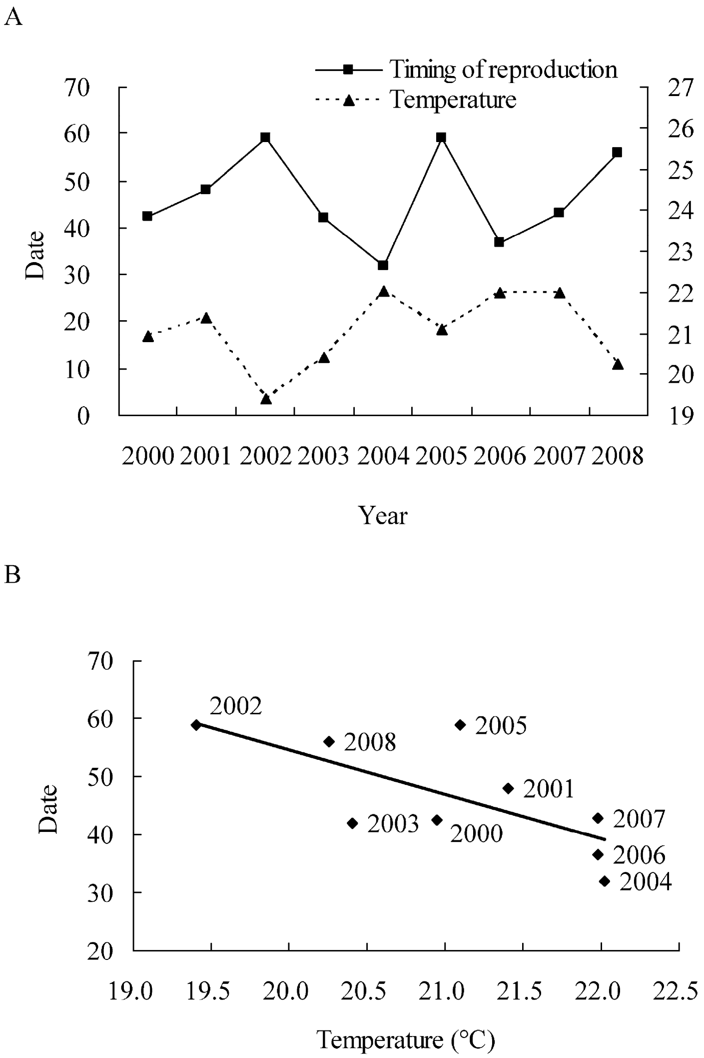

圖 3. 2000-2008 年領角鴞繁殖起始時間與前一年 11 月均溫之關係圖(A)及迴歸圖(B)，日期是以每年 1 月 1 日起的天數表示。

Fig. 3. Annual egg deposition dates of the Collard scops owl in 2000-2008 in correspondence to monthly average air temperatures of November of the previous year (A), and their linearly regressive relationship (B); Date=accumulated days from January 1.

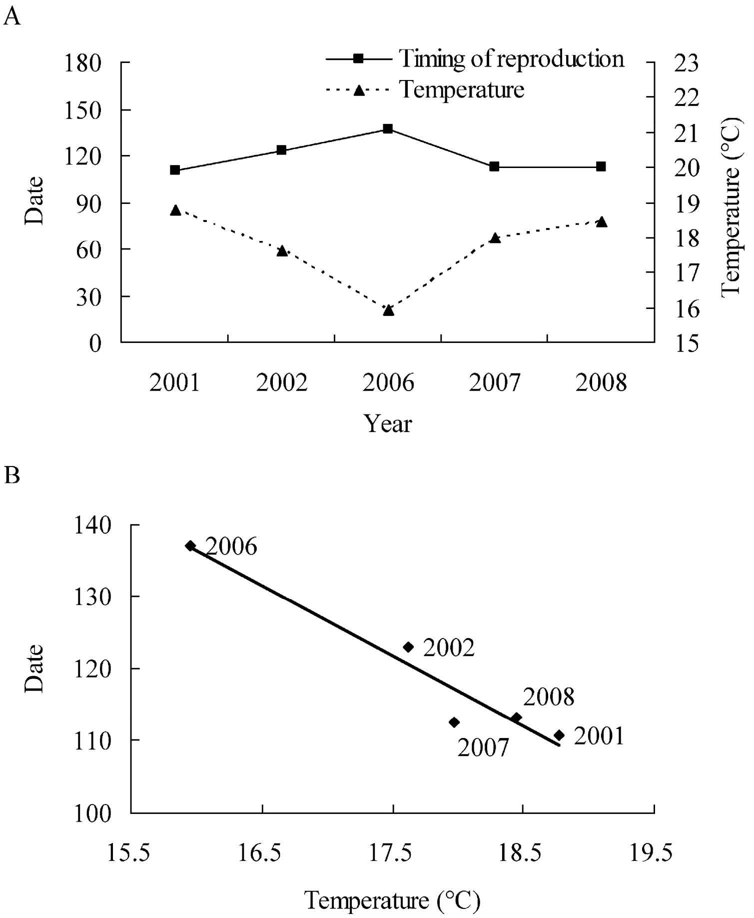

圖 4. 2001、2002、2006-2008 年黑冠麻鷺繁殖起始時間與前一年 12 月均溫(A)及迴歸圖(B)，日期是以每年 1 月 1 日起的天數表示。

Fig. 4. Annual egg deposition dates of the Malayan night heron in 2001, 2002 and 2006-2008 in correspondence to monthly average air temperatures of November of the previous year (A), and their linearly regressive relationship (B); Date=accumulated days from January 1.

討 論

利用落巢案例推算出的領角鴞產卵時間從 12 月到隔年 7 月，長達 8 個月的時間，較林(2003a)在中部森林觀察所提出的 3-7 月來得長，原因可能與部分案例來自都會區有關，繁殖高峰期落在 3 月份，則與林(2003a)的資料相符，次爲 4 月和 2 月。黑冠麻鷺產卵時間從 3 月到 8 月，高峰期出現在 4 月份，與姚(2002)的研究結果相同。

研究顯示某些鳥類在都會區的繁殖時間早於原始棲地，因爲都會區的食物較易獲得(Fleischer Jr. *et al.* 2003)，而台灣中部地區的領角鴞也有類似現象(林 2003b)。由於急救站的落巢幼鳥均爲民眾送交，病歷上僅記載概略地點，無法得知確實的巢位環境，若以大範圍的城鄉行政區劃分，28.5%的領角鴞巢位來自人口密度高於 1,000 人/km^2 的都會區，2.3%來

自 100 人/km² 以下的原始環境，其餘 69.2%是來自都會區與原始環境間的過渡地帶，即使受都會化影響的程度不同，其差異也不明顯，因此領角鴞各年度繁殖起始時間與都會區案例的比例並無顯著趨勢。事實上在原始環境中落巢的幼鳥被民眾發現並送往急救站的機率並不高，本研究認爲急救站的落巢幼鳥案例大多數都是來自於人類活動較頻繁的地區。

本研究的前提假設是落巢案例的頻度可以反映野外動物的繁殖週期，但是在樣本數不夠多的情況下，可能無法將繁殖週期完整的呈現。本研究的繁殖高峰期雖和野外的資料一致(姚 2002; 林 2003a)，但在離峰時期也許會有所遺漏，無法精準的代表野外動物繁殖的起始時間，然而若以較巨觀的角度，看不同年間較早或較晚繁殖的趨勢，則確實跟特定月份的平均溫度走勢有顯著關聯。由於領角鴞和黑冠麻鷺在條件允許下，一年可以繁殖兩次(姚 2002; 林 2003a)，導致繁殖季拉長，本研究分別以前 1/3 和前 1/2 巢數平均的產卵時間作爲繁殖起始時間，可避免將第二次繁殖的巢納入計算。

領角鴞繁殖高峰出現在 3 月份，與繁殖起始時間最相關的月均溫爲 11 月，而黑冠麻鷺繁殖高峰爲 4 月份，繁殖起始時間與 12 月均溫最相關，顯示繁殖高峰之前 3 個月的溫度，已可能會對鳥類繁殖造成影響。研究顯示某些候鳥春季北返和繁殖的時間確實會受到數個月前冬季溫度的影響(Sillett *et al*. 2000; Saino *et al*. 2004)，2004-2005 年在太魯閣國家公園的研究中，便發現 4 月份繁殖的煤山雀(*Parus ater*)在 2005 年最早產卵日較前一年早了 11 天，可能是因爲 2005 年 1 月的溫度高於 2004 年(蕭等 2008)。

其他月份均溫雖與繁殖起始時間的相關程度未達到顯著性，並不代表對繁殖時間就沒有影響，例如領角鴞 2005 年的繁殖起始時間，若以 2004 年 11 月的均溫代入本研究的迴歸式計算，應爲 46 天(即 2 月 15 日)，但依案例所計算出的天數則爲 59 天(2 月 28 日)，兩者的差距頗大(圖 3b)，可能是因爲 2004 年 12 月到 2005 年 2 月的平均溫度較其餘年份的同期低(圖 5)，使得 2005 年的繁殖起始時間延後。因此影響領角鴞繁殖時間的均溫月份或許可分爲主要(前一年的 11 月)與次要(前一年 12 月至當年 2 月)，若未來進行預測模式時應一併納入考量。

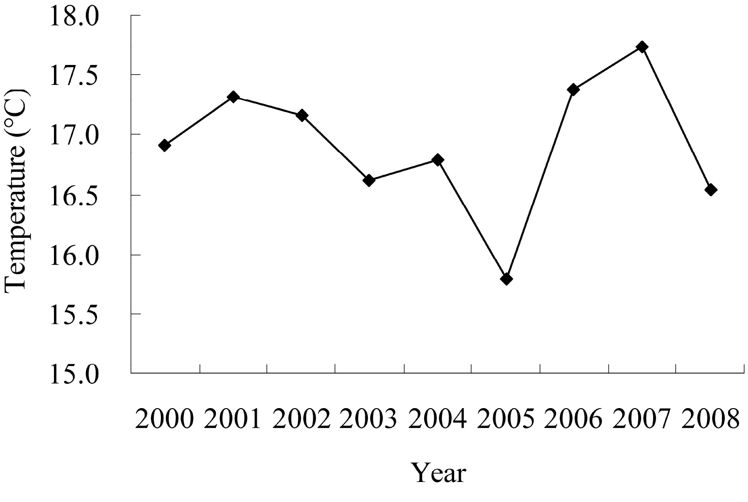

圖 5. 2000-2008 年 12-2 月的平均氣溫變化。

Fig. 5. Average air temperatures of the months of December-February, 2000-2008.

Stevenson and Bryant (2000)指出基於生理因素，各個物種對暖化反應的程度不一致，其中體型小的鳥類受暖化影響的程度較大。國際間已發表許多鳥種繁殖時間和相關月份溫度的迴歸式，多數爲燕雀目(Order Passeriformes)的小型鳥類，當特定月份均溫升高 1°C，提前繁殖的天數約 1.5-3.5 天(表 6)。領角鴞和黑冠麻鷺的體型較燕雀目鳥類大得多，提前繁殖的天數卻分別達到 7.7 和 9.7 天，是國際間研究結果的 2-3 倍，是否此 2 物種對溫度的敏感性較高，或是本研究的誤差較大，有待野外研究來證實。研究顯示台灣氣候暖化幅度以近 25 年較大，且冬季增溫幅度較明顯(林 2005)，更有可能加強台灣鳥類提前繁殖的程度。

表 6. 國外相關研究中特定月份溫度每升高 1°C 繁殖時間會提前的鳥種和天數

Table 6. Numbers of days to breed earlier with increasing air temperature of 1°C in specific months for different bird species in literature

Geographical area	Species	Months	Days	References
North America	Tree Swallow (*Tachycineta bicolor*)	May	3.49	Dunn and Winkler 1999
Netherlands	Northern lapwing (*Vanellus vanellus*)	16 Feb-15 Mar	1.64	Both *et al*. 2005
Croatia	Marsh tit (*Parus palustris*)	Feb-Apr	2.08	Dolenec 2006
Croatia	Blue tit (*Parus caeruleus*)	Feb-Apr	2.09	Dolenec 2007
Italy	Swift (*Apus apus*)	April	2.89	Rubolini *et al*. 2007
Estonia	Common gull (*Larus canus*)	31 Mar-26 Apr	1.43	Brommer *et al*. 2008

一般而言，溫度變暖對鳥類繁殖有益，提前繁殖使幼鳥在寒冷的冬季來臨前有更多的成長時間(Crick *et al*. 1997)，部分鳥類則顯示出孵卵期縮短(Ardia *et al*. 2006)、產卵數增加以及繁殖成功率提升(Winkel and Hudde 1997; Schaefer *et al*. 2006)，但前提是要跟鳥類賴以維生的食物物候週期改變的趨勢相同(Møller 2008)。若食物鏈中各個物種因溫度變化而改變物候週期的程度不一致，就會導致食物供給失衡，例如在荷蘭某處森林中，鱗翅目(Order Lepidoptera)幼蟲發生的高峰期因暖化而提前，白頰山雀(*Parus major*)的繁殖週期卻未能跟上(Visser *et al*. 1998)，不少類似的案例造成鳥類繁殖成功率下降、延後繁殖和產卵數減少(Sazn 2003; Laaksonen *et al*. 2006)。學者預期整個生態系物候週期的長期趨勢都會提前，但物種間相互關係的重新調整將會付出極大的代價(Parmesan and Yohe 2003; Visser *et al*. 2003)，稀有種也將面對更大的生存壓力(Crick 2004)。

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台灣中部黑枕藍鶲之鳥巢掠食風險與巢位特徵

Nest-Site Characters Related to Predation Risk for the Black-Naped Monarch (*Hypothymis azurea*) in West-Central Taiwan

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摘　要

鳥巢天敵是威脅鳥類能否繁殖成功的重要因素之一。本研究我們假設鳥巢覆蓋度、巢樹大小、鳥巢可接近性、巢枝支撐度、巢樹類型、水源及人類活動頻度會影響黑枕藍鶲(*Hypothymis azurea*)的鳥巢天敵掠食風險。2007-2008 年繁殖季，我們於台灣中部雲林縣東側以竹林及次生闊葉林爲優勢植被的丘陵地，尋找黑枕藍鶲鳥巢，隨後監測發現之鳥巢以追蹤各巢最終繁殖命運。繁殖成功(以有 1 隻或以上幼鳥成功離巢定義之，n = 22)及被天敵掠食鳥巢(n = 27)間的特徵差異以邏輯迴歸分析檢測。以訊息理論研究法(AIC*c*)進行模式比較的結果顯示巢可接近性及巢樹大小，分別由巢距冠層邊緣最近距離及巢樹高代表，最爲研究資料所支持(累積ωi = 0.785)。另由 Odd ratios 來看，巢距冠層邊緣距離每增加 1m，繁殖成功率上升約 87%，而巢樹高度每增加 1m，則提升約 18%。研究結果顯示黑枕藍鶲透過將巢築在不容易接近及(或)搜索的位置來降低天敵掠食的風險。

Abstract

Nest predation is one of the most sever threats to the breeding success of passerine birds. We examined nest-site characters (variables), such as nest covers, nest inaccessibility, nest supported plants (species, sizes and stem strength), and distances to water sources and human activities, for the black-napped monarch (*Hypothymis azurea*) in a hill area covered predominantly with cultivated bamboos and secondary broad-leaf trees in the west-central Taiwan. We tracked nesting success, quantified their outcomes, and determined the causes of the nesting failures for two breeding seasons in 2007-2008. We compared nest-site variables between succeed nests (fledged at least one offspring, n = 22) and depredated nests (n = 27) with binary logistic regression. The results of model comparisons with Akaike's information criterion (AIC*c*) showed that nest inaccessibility and plant sizes expressed, respectively, as distances of the nests from foliage edges and plant heights were found to be the most important variables determining the nesting success (accumulated ωi = 0.785). Based on the odds ratios, an increase in 1m each of the distance and the height increased the breeding success of 87% and 18%, respectively. Apparently, the black-naped monarch makes nests at inaccessible sites to avoid predation.

關鍵詞：黑枕藍鶲、繁殖成功率、巢位特徵、鳥巢天敵、台灣

Key words: *Hypothymis azurea*, nesting success, nest-site characters, nest predators, Taiwan

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緒　言

鳥類族群的存續與繁殖成功率的高低息息相關(Newton 1998)，而在眾多影響繁殖成功率的因子中，巢中鳥蛋及幼雛遭天敵捕食，已知是影響燕雀目鳥類能否繁殖成功的主要原因之一(Ricklefs 1969; Martin and Roper 1988)。此外，食物資源的豐富度及可利用性、適合巢位的有無及人爲干擾的嚴重性，也都會直接、間接地影響鳥類的繁殖成功(Soulé *et al*. 1988; Robbins *et al*. 1989; Canterbury and Blockstein 1997; Sasvári and Hegyi 1998)。因此，鳥類爲了提高自身的繁殖成功率，會傾向選擇於食物資源豐富、人爲干擾不多、支撐穩固、同時天敵又不易發現或接近的地點來築巢。

植物結構的複雜度，特別是緊鄰巢旁的植物覆蓋度經常被認爲與鳥巢天敵的捕食率有關，如巢隱蔽度假說(nest-concealment hypothesis)即預測鳥巢旁的植物覆蓋愈密愈可以藉由阻礙天敵的視覺、嗅覺或聽覺等感官能力，來降低鳥巢被發現的機會(Martin 1993)。 而另一個類似的假說，潛在巢位數假說(potential-prey-site hypothesis)則預期築巢於一潛在巢位數量較多的環境，天敵需搜尋較多地點，因而鳥巢被找到的機率較低(Bowman and Harris 1980; Martin 1993)。而對在樹冠築巢的燕雀目鳥種而言，大樹應可提供較多的潛在巢位。此外，巢位的隱蔽程度也與巢上方覆蓋度有直接關係(Eggers *et al*. 2006)，而主幹的胸高直徑影響樹的覆蓋度，通常胸高直徑愈大的樹，其樹愈高、樹冠層的覆蓋度愈大，相對的隱密度也比較高，可提供鳥巢較好的隱蔽效果(Alonso *et al*. 1991)。

除了是否容易被發現外，鳥巢是否易於接近，亦可以是影響天敵掠食機率的重要因素。如對蛇類及哺乳類天敵而言，巢距地面或主幹較遠，以及巢樹主幹很直或分枝較多都可能增加天敵上爬或接近巢的難度(Thibault *et al*. 2002; 陳等 2005)；但對來自空中的天敵而言，遠離樹冠邊緣的比較不容易被發現及接近(Alonso *et al*. 1991)。故若地面掠食者是鳥巢主要威脅時，親鳥常會把巢築在遠離地面及距離主幹較遠處(Alonso *et al*. 1991; Thorstrom and Quixchán 2000; Kershner *et al*. 2001)；而考量到空中掠食者的掠食時，則遠離樹冠層邊緣是可行的策略(Alonso *et al*. 1991)。支撐鳥巢的微結構，如樹枝大小及強度，除了惡劣天候及支撐重量的考量外，巢枝粗細也會影響哺乳類及爬蟲類掠食者接近巢的機會，如果巢枝的強度無法支撐掠食者體重的話，則掠食者無法有效的搜尋或掠食鳥巢(Hamao 2005)。另外，巢樹種類也可能是鳥類築巢的考量重點之一。某些常被用來築巢的樹種也許具備特殊的形態或構造，而可降低天敵的捕食，但也有很多鳥類對巢樹種類並無特定偏好，其考量的重點爲能否提供巢位最佳隱蔽度以避開天敵的樹種或植被(Belles-Isles and Picman 1986; Alonso *et al*. 1991; Ueta 1998; Liebezeit and George 2002)。

鳥巢附近的人類活動頻度，也常是影響天敵組成或數量的原因。人類活動經常導致森林棲地碎裂化及邊緣環境的增加，此種改變，除了可能影響食物資源的變化外，也常會導致掠食者種類變多或數量增加，進而提高鳥巢的被掠食率(Millers and Knight 1993; Götmark *et al*. 1995; Smith *et al*. 1999; Miller *et al*. 2003; Chace and Walsh 2004; Small 2005)。然而，也有些鳥種因食性、天敵分布、有特殊需求或對破碎化棲地容忍度較高，在開發程度較高或都市化地區，反而因壓力的釋放或新棲地的增加，而成爲該類棲地的優勢族群(Andrén 1992; Burger and Gochfeld 1998; Smith *et al*. 1999; Dykstra *et al*. 2000; Piper and Catterall 2004)。此外，也有研究發現巢位附近是否有穩定的水源，也會與植被結構的複雜度和繁殖所需的昆蟲量有關，進而影響繁殖成功率或窩蛋數(Bekoff *et al*. 1987; Atlegrim 1992; Suarez *et al*. 1997; 王等 2006)，但水源是否與天敵活動有關，則較少有研究探討。

黑枕藍鶲(*Hypothymis azurea*)屬於雀形目(Passeriforms)王鶲科(Monarchidae)，廣泛分布於南亞及東南亞，出現於台灣的黑枕藍鶲(*H. a. oberholseri*)屬台灣特有亞種(Coates *et al*. 2006)。黑枕藍鶲爲台灣平地至中海拔(< 1,600m)普遍的留鳥(翟 1977)，棲地適應亦相當廣泛，只要有大面積樹木連續生長之處，都有出現的機會(王等 1991)。其個性機警好動，通常單獨或成對在樹林中層活動，以昆蟲爲主食。另其繁殖季在 4-7 月間(李 1986)，喜在樹冠中下層的細小枝椏或藤蔓間築杯形巢(周 1995)。雖然黑枕藍鶲數量普遍分布亦廣，但目前仍無巢位與天敵掠食風險關係的相關探討，本研究即以其爲對象並分別檢測鳥巢覆蓋度、巢樹大小、鳥巢可接近性、巢枝支撐度、巢樹類型、水源及人類活動頻度等因子，是否在黑枕藍鶲的巢位棲地特徵與天敵風險關係中獲得支持及其關係爲何。

材料與方法

一、研究地點

本研究於雲林縣林內鄉及斗六市東側的丘陵地，即湖本野生動物重要棲息環境的範圍及其周邊(圖 1)，研究地區海拔高度介於 50-200m 之間，氣候屬於夏季炎熱潮濕、冬季乾涼的亞熱帶氣候型態，5 月至 10 月爲濕季，雨量約爲 1,800 mm，乾季則從 11 月至翌年 4 月，雨量常少於 300 mm (廖 2001; 陳 2007)。

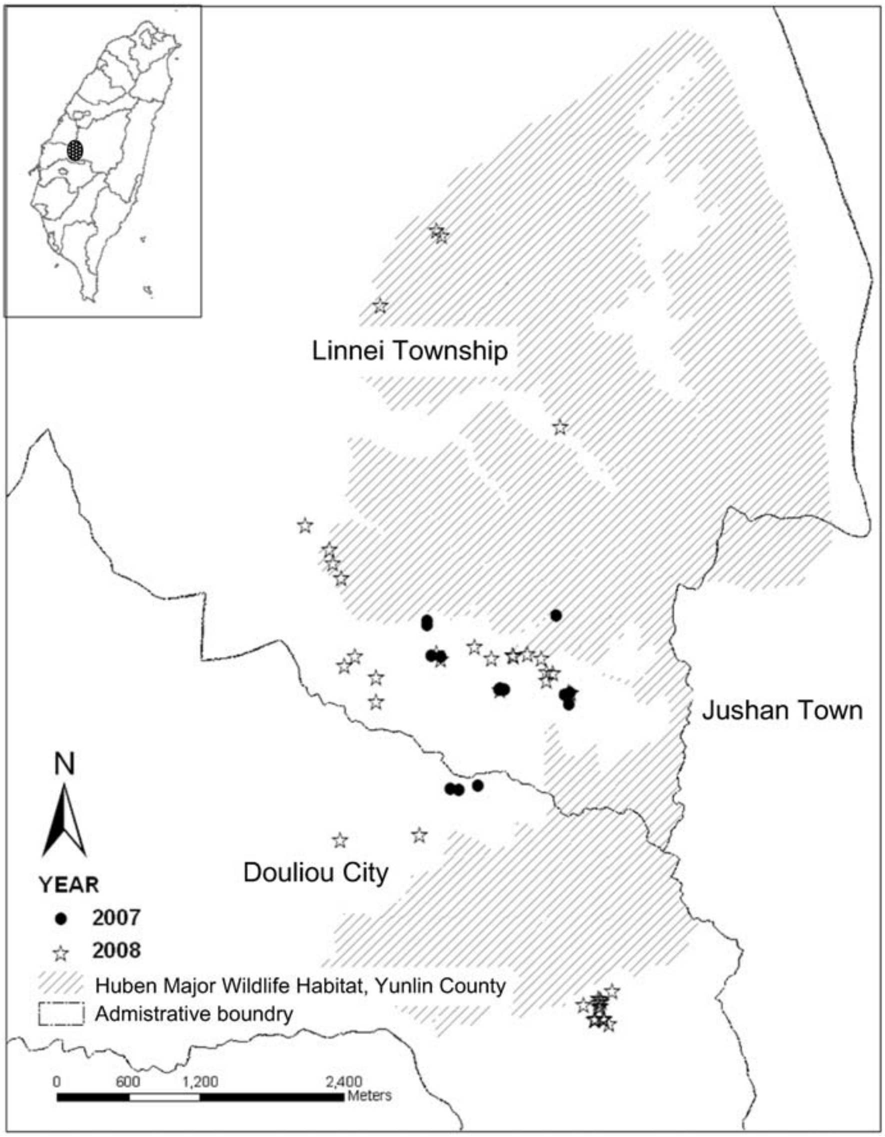

圖 1. 2007-2008 年繁殖季於雲林縣林內鄉及斗六市記錄的黑枕藍鶲(*Hypothymis azurea*)鳥巢的空間分布。

Fig. 1. Spatial distribution of black-naped monarch (*Hypothymis azurea*) nests recorded in the breeding seasons of 2007-2008 in Linnei Township and Doulieu City, Yunlin County, west-central Taiwan.

研究地點的環境植被組成以人爲種植的麻竹(*Dendrocalamus latiflorus*)、龍眼(*Euphoria longana*)及柳丁(*Citrus sinensis*)等農作物爲主。此區爲人類活動頻繁的區域，在人爲干擾較少的溪谷與集水區上游的優勢樹種有血桐(*Macaranga tanarius*)、山黃麻(*Trema orientalis*)、稜果榕(*Ficus septica*)、水同木(*Ficus fistulosa*)、香楠(*Machilus zuihoensis*)等次生闊葉樹；而藤本植物以風藤(*Piper kadsura*)、糙莖菝葜(*Smilax bracteata*)及柚葉藤(*Pothos chinensis*)爲主，林下最常見的小灌木則是構樹(*Broussonetia papyrifera*)及山棕(*Arenga engleri*)；草本則以龍船花(*Clerodendrum kaempferi*)、青莧(*Amaranthus patulus*)、觀音座蓮(*Angiopteris lygodiifolia*)及小毛蕨(*Christella acuminata*)爲主。

二、尋巢、探巢及鳥巢命運的判定

於 2007 年及 2008 年的 3-8 月，由 1-2 位調查人員在樣區內沿著村落道路、產業道路、河床、果園、山坡地，藉由觀察及追蹤成鳥任何與繁殖有關的行爲，如警戒、求偶、咬巢材及口啣食物等，另亦注意幼鳥索食聲，來搜尋巢位的所在地。找到正在築巢或使用中的鳥巢後，每隔 2-3 日巡巢 1 次，記錄日期、蛋或雛鳥數量、親鳥行爲及離巢幼鳥數等繁殖狀況，直到幼鳥成功離巢或繁殖失敗爲止。爲避免探巢的干擾導致親鳥棄巢或巡巢過程引來天敵(Morton *et al*. 1993)，先在距離鳥巢約 5-10m 處使用望遠鏡觀察，避免直接碰觸鳥巢，若親鳥不在巢，即以探巢器(綁在 6m 伸縮桿上的圓形小鏡片)確認巢內的狀況。每次探巢時間控制在 5-10 min 之間。若巢太高無法以探巢器查看時，即於 5-10m 外持續監看 30 min，如仍不見親鳥回巢，則先行探視其他各巢，最後再返回觀察，若仍不見親鳥回巢，隔日再探巢，連續 3 日。各巢只要有 1 隻雛鳥離巢，即視爲繁殖成功。繁殖成功或失敗以最後監測日期及最後一次監測當時巢內外狀況做爲判別的依據，判斷標準如下：當(一)最後一次探巢時至少有 1 隻雛鳥離巢；(二)在巢外看見或聽見雛鳥；(三)最後一次探巢時雛鳥的羽毛發育已接近離巢時的狀態都判定爲繁殖成功。而當(一)於繁殖期間鳥巢被扯壞毀損或親鳥不再使用；(二)在可能離巢日之前蛋或雛鳥全部消失；(三)巢內發現碎蛋殼、雛鳥屍體或大量羽毛則判定爲繁殖失敗(Manolis *et al*. 2000)。失敗的巢再依據可能造成的原因區分爲天敵掠食、氣候與其他等 3 類，區別的標準爲：(一)有前述明顯掠食跡象或整窩蛋、雛鳥在可能離巢日之前消失爲被天敵掠食；(二)當豪雨、強風或颱風過後鳥巢掉落或在巢中或巢外發現尚未孵化的蛋或雛鳥屍體則視爲氣候因素；(三)非以上原因失敗的鳥巢歸類爲其他因素。

三、巢位測量

爲了避免因距離繁殖結束的時日過久，導致巢位特徵的改變，對幼鳥離巢後或判定繁殖失敗的鳥巢，於各巢結束使用後的 2 週內對下列 11 項棲地因子進行測量，包括：(一)巢樹種類(NST, plant species)，記錄樹種名稱，若非樹木或無法確認種類時，記錄其類型；(二)巢樹高度(NSBSHT, plant height, cm)；(三)巢樹主幹胸高直徑(DBH)，測量巢樹主幹離地 130 cm 處的胸高直徑(cm)；(四)鳥巢上方樹冠層覆蓋度(COVER, canopy cover)，站在鳥巢正下方，以覆蓋度測量儀(densiometer, Lemmon 1957)分別測量鳥巢正上方東、西、南、北四個方位的樹冠層覆蓋度(%)，再取平均值；(五)巢枝直徑(DSPBR, diameter of support branches)，測量支撐鳥巢的枝椏直徑(mm)；(六)與主幹的距離(DSTEM, distance from stem)，即鳥巢至巢樹主幹間的最短水平直線距離(cm)；(七)巢與冠層邊緣的距離(DSEDG, distance of nest from foliage edge)，即鳥巢至上方樹冠層邊緣的最近距離(cm)；(八)巢與地面的距離(NSTHT, nest height)，即鳥巢底部至地面的垂

直距離(cm)；(九)巢與最近水源的距離(DSWAT, distance to nearest water)，即鳥巢所在位置至永久性水源的最近水平距離(m)；(十)巢與最近道路的水平距離(DSROAD, distance to nearest road, m)；以及(十一)巢與最近建築物的水平距離(DSBULD, distance to nearest building, m)。

四、資料分析

本研究利用邏輯迴歸(logistic regression) (Hosmer and Lemeshow 2000)來分析成功繁殖與被天敵掠食的黑枕藍鶲鳥巢在巢位特徵上的差異，並利用訊息理論研究法(information-theoretic approach) (Burnham and Anderson 2002)進行巢位特徵與天敵掠食風險關係的模式比較，以尋找出最爲資料所支持的模式。另由於繁殖資料蒐集自 2 個年份，且各巢繁殖起始時間(下第 1 顆蛋的日期)有所不同，故先檢視此 2 變數對繁殖成功是否有顯著影響。

透過文獻回顧以及研究者對黑枕藍鶲的認識，本研究建立以下 7 個可能影響鳥巢天敵掠食風險的候選模式，以下爲各模式及其包含的變數：

(一)鳥巢覆蓋度(nest cover)：鳥巢上方樹冠層覆蓋度(COVER)。

(二)巢樹大小(plant size)：巢樹高度(NSBSHT)、巢樹主幹胸高直徑(DBH)。

(三)鳥巢可接近性(nest inaccessibility)：巢與主幹的距離(DSTEM)、巢與冠層邊緣的距離(DSEDG)及鳥巢與地面的垂直距離(NSTHT)。

(四)巢枝支撐度(support strength)：巢枝直徑(DSPBR)，區分爲主枝(DSPBR1)、分枝(DSPBR2)與平均直徑(DSPBRM)。

(五)巢樹類型(plant species, NST)：主要檢測樣區最優勢的竹類和其他植物的差別，故巢樹類型區分爲竹類(以麻竹爲主)及非竹類。

(六)水源(water resource)：巢與最近水源的距離(DSWAT)。

(七)人類活動頻度(human activity)：以巢與最近道路的水平距離(DSROAD)及巢與最近建築物的水平距離(DSBULD)爲指標。

由於各模式中的變數可能存在高相關性而致有共線性(multicollinearity)問題，故先以 Pearson’s product-moment correlation coefficient 檢測其相關性，若同一模式中的變數相關性過高(如 > 0.6)，則先以單變數邏輯回歸分析各變數對繁殖成功與否的影響，並僅選擇影響較大者(即 p 值較小者)於模式中。之後，再以 Box-Tidwell Transformation test (Tabachnick and Fidell 2001)檢測各變數是否符合邏輯回歸之前提，即 logit 值須是線性的。

模式選擇以校正樣本數後的 Akaike’s Information Criterion 分數(AIC*c*)、Akaike’s Information Criterion 分數差值(ΔAIC*c*)及 Akaike weights (ωi) (Burnham and Anderson 2002)爲標準。此方法在進行時，先計算各模式的 AIC*c* 值及各模式與具有最低 AIC*c* 值模式的 AIC*c* 差值(ΔAIC*c*)，之後係以 ΔAIC*c* ≤ 2 的模式作爲最被資料所支持的模式(Burnham and Anderson 2002)。此外，以 Akaike weights (ωi)來評估一特定模式在同樣的資料及一組相互比較的模式中是否爲最佳模式(Burnham and Anderson 2002)。一旦模式被選擇後，再以 Likelihood ratio test 進一步瞭解其對繁殖成功與否的貢獻，以追求模式的最簡約化(parsimony)，而一旦發現有變數得以移出原最佳模式，即再重新計算其 AIC*c*、ΔAIC*c* 及 ωi 等數值，以比較模式選擇間的變化。另以 Hosmer-Lemeshow goodness of fit test 檢測最佳模式的適合度(Hosmer and Lemeshow 2000)；統計值不顯著($p > 0.05$)代表模式可被接受。另計算 Odds ratios 及 95%信賴區間(confidence intervals, CI) (Hosmer and Lemeshow 2000)，以進一步評估各變數的影響。所有統計分析均以 SAS, version

9.0 (SAS Institute Inc. 2002)進行。

結果

2007年尋獲有使用的黑枕藍鶲巢15個，2008年43個，2年共計58個，其中大埔溪集水區43個、北勢坑溪上游15個(圖1)。2年合計繁殖成功的巢有22個(38%)，繁殖失敗的巢爲36個(62%)。繁殖失敗的主因爲遭天敵掠食(27個，75%)，天氣因素次之(5個，13.9%)，其他因素則有4個(11.1 %)。不同年度及下第1顆蛋的日期對繁殖成功或失敗沒有顯著影響(Logistic regression test; 年度，df = 1, $x^2 = 0.175$, $p = 0.675$; 下蛋日，df = 1, $x^2 = 1.17$, $p = 0.279$)。

各模式經以相關分析、單變數分析及檢測是否符合邏輯回歸分析的前提後，包含的變數的組成如表1。以訊息理論研究法進行模式比較，發現鳥巢可接近性($\Delta\mathrm{AIC}c = 0$)及巢樹大小模式($\Delta\mathrm{AIC}c = 1.63$)的$\Delta\mathrm{AIC}c \leq 2$，其累積的Akaike weights ($\omega i$)爲0.613 (表1)。再以likelihood ratio test檢測2模式在移除任一變數後，其模式表現是否有顯著改變，發現巢樹大小模式於移除胸高直徑(DBH) (likelihood ratio test; $df = 1$, $x^2 = 0.29$, $p = 0.592$)及鳥巢可接近性模式於移除巢高(NSTHT) (likelihood ratio test; $df = 1$, $x^2 = 0.92$, $p = 0.338$)後，對於模式表現並無顯著影響，故再以簡約後的7個模式重新以訊息理論研究法進行模式比較。此時，以巢與冠層邊緣距離(DSEDG)爲代表的鳥巢可接近性模式($\Delta\mathrm{AIC}c = 0$)及以巢樹高度(NSBSHT)爲代表的巢樹大小模式($\Delta\mathrm{AIC}c = 1.01$)的累積$\omega i$提高爲0.785 (表2)。以Hosmer-Lemeshow test檢測顯示2模式均與資料相符合(鳥巢可接近模式，$df = 6$, $x^2 = 2.67$, $p = 0.849$; 巢樹大小模式，$df = 7$, $x^2 = 4.6738$, $p = 0.7$)。

最終分析結果顯示鳥巢可接近性模式及巢

表 1. 以訊息理論研究法(AIC*c*)對黑枕藍鶲(*Hypothymis azurea*)的鳥巢被掠食風險與巢位特徵關係進行模式選擇之結果；表中同時顯示變數數量(*K*)、AIC*c*值、AIC*c*差值($\Delta\mathrm{AIC}c$)及Akaike weights (ωi)

Table 1. Ranking of models of individual nest-site variables for the nesting successes of the black-naped monarch (*Hypothymis azurea*) based on the smallest AIC*c* value ($\Delta\mathrm{AIC}c$) (*K*, number of variables in the models; ωi, Akaike weight)

Models	Variables[a]	*K*	AIC*c*	$\Delta\mathrm{AIC}c$	ωi
Nest inaccessibility	DSEDG, NSTHT	3	69.670	0	0.425
Plant size	NSBSHT, DBH	3	71.310	1.640	0.187
Support strength	DSPBRM	2	72.621	2.951	0.097
Nest cover	COVER	2	72.706	3.037	0.093
Water resource	DSWAT	2	73.075	3.405	0.078
Plant species	NST[b]	2	73.421	3.751	0.065
Human activity	DSROAD, DSBULD	3	73.798	4.129	0.054

[a] Variables abbreviations: plant species (NST), plant height (NSBSHT), diameter at breast height (DBH), canopy cover (COVER), mean diameter of support branches (DSPBRM), distance of nest from foliage edge (DSEDG), nest height (NSTHT), distance to nearest water source (DSWAT), distance to nearest road (DSROAD) or to nearest building (DSBULD).

[b] The nesting plant tree species were categorized as bamboo and non-bamboo.

表 2. 以訊息理論研究法(AIC*c*)對黑枕藍鶲(*Hypothymis azurea*)簡化變數後的巢位特徵與天敵掠食風險關係進行模式選擇之結果；表中同時顯示變數數量(*K*)、AIC*c* 值、AIC*c* 差值(*Δ*AIC*c*)及 Akaike weights (*ωi*)

Table 2. Ranking of reduced models of individual nest-site variables for the nesting successes of the black-naped monarch (*Hypothymis azurea*) in Yunlin County, Taiwan, 2007-2008, based on the smallest AIC*c* value (*Δ*AIC*c*) (*K*, number of variables in the models; *ωi*, Akaike weight; variables evaluated with likelihood ratio test before model reduction)

Models	Variables	*K*	AIC*c*	*Δ*AIC*c*	*Ωi*
Nest inaccessibility	DSEDG	2	68.214	0	0.489
Plant size	NSBSHT	2	69.220	1.007	0.296
Support strength	DSPBRM	2	72.621	4.407	0.054
Nest cover	COVER	2	72.706	4.493	0.052
Water resource	DSWAT	2	73.075	4.861	0.043
Plant species	NST	2	73.421	5.207	0.036
Human activity	DSROAD, DSBULD	3	83.601	5.585	0.030

樹大小模式最爲本研究資料所支持，其各自包含的變數爲巢距冠層邊緣的距離(DSEDG)及巢樹高度(NSBSHT)，且 2 變數對黑枕藍鶲繁殖成功與否均有顯著正向影響(表 3)。另由 Odd ratios 來看，巢距冠層邊緣距離(DSEDG)每增加 1m，繁殖成功率上升約 87%，巢樹高度(NSBSHT)每增加 1m，繁殖成功率也會提升約 18% (表 4)。

表 3. 以 AIC*c* 選擇出黑枕藍鶲(*Hypothymis azurea*)巢位最佳模式的 Logistic regression 模式變數的估計值、SE 及其檢定結果

Table 3. Results of logistic regression analysis for the black-naped monarch (*Hypothymis azurea*) nesting success models best supported by AIC*c*

Models	Variables	Coefficients	SE	Chi-square	*P*
Nest inaccessibility	Intercept	-1.40	0.62	5.15	0.023
	DSEDG	0.63	0.29	4.77	0.029
Plant size	Intercept	-1.34	0.63	4.49	0.034
	NSBSHT	0.16	0.08	4.12	0.043

表 4. 對黑枕藍鶲(*Hypothymis azurea*)繁殖成功與否最具影響的最佳模式(*Δ*AIC*c* $\leq$ 2)中，各變數的勝算比(Odds ratio)與其 95%信賴區間(Confidence Interval, CI)

Table 4. Odds ratios and 95% confidence intervals (CI) for the nest-site variables of the black-naped monarch (*Hypothymis azurea*) with the best nesting success models supported by AIC*c*

Models	Variables	Odds ratio	CI
Nest inaccessibility	DSEDG	1.872	1.066 - 3.286
Plant size	NSBSHT	1.179	1.006 - 1.383

討　論

簡化後的可接近性模式及巢樹大小模式，最能解釋繁殖於雲林縣林內鄉及斗六市丘陵環境中的黑枕藍鶲鳥巢遭天敵掠食的風險，其代表變數分別爲巢距冠層邊緣的距離(DSEDG)及巢樹高度(NSBSHT)，且 2 變數都與能否繁殖成功，即逃離天敵掠食，有顯著的正向關係，也就是說鳥巢若距離樹冠層邊緣愈遠及鳥巢所在的巢樹愈高，繁殖成功的機率會向上提升。其餘 5 個同時競爭的模式，包括：人類活動頻度、巢支撐度、巢樹類型、巢覆蓋度及水源模式，都不爲本研究的資料所支持。

本研究所有繁殖失敗的黑枕藍鶲鳥巢中，有 75%肇因於天敵掠食，顯示黑枕藍鶲與大部分燕雀目鳥類相同，在繁殖時會面臨強大的天敵壓力(Ricklefs 1969; Martin 1993)。探討巢位棲地特徵與天敵掠食機率關係的研究眾多，然而，在尋求降低鳥巢被天敵發現的共同選汰壓力下，各鳥種因其自身的生物習性及居住的棲地特性，會展現出不同的選擇策略，故在討論特定鳥類的巢位特徵與天敵壓力的關係時，必須仔細分析該生物本身具備的特性和研究地區的天敵組成。

黑枕藍鶲不僅爲台灣中低海拔(< 1,600m)普遍的留鳥(翟 1977; 葛及李 2003)，且其棲地雖以森林爲主，但可廣泛適應於闊葉林、次生林、林緣、樹林及公園等多樣環境(王等 1991)，同時於樹林上層到下層都可活動(Coates *et al*. 2006)。另其性情活躍而領域性強烈，常發出叫聲，故明顯而容易覺察(Goodale and Kotagama 2005; Coates *et al*. 2006)。黑枕藍鶲經常選用直立岔枝來築巢，巢本身常缺乏僞裝及遮蔽，故不難爲人所發現(Coates *et al*. 2006)。繁殖期間常追擊(mobbing)或騷擾(harassing)其他可能威脅鳥巢的鳥類或天敵(Coates *et al*. 2006)。整體而言，黑枕藍鶲的築巢習性與許多其他王鶲科的鳥類相當類似，在緊鄰鳥巢的地方普遍缺乏掩蔽，故可能因而導致此科鳥種的繁殖成功率偏低，唯王鶲科成鳥的壽命常在 15 年以上(Coates *et al*. 2006; VanderWerf 2008)，也許其策略爲減少各年的投資，降低自身的壽命威脅，故其一生的適存度(fitness)不見得比較低。

雲林縣林內鄉及斗六市的低海拔丘陵地區，其目前的棲地型態係以人爲種植的麻竹、龍眼及柳丁等農作物爲主，次生闊葉樹則交雜在較難開墾的地點及荒廢林地之中，住家則沿著主要溪谷分布。陳(2007)曾探討本研究相同範圍內灌叢巢及地面巢的天敵種類，發現灌叢巢的天敵種類包括蛇類、鳥類、哺乳類及螞蟻等，其中以大頭蛇(*Boiga kraepelini*)、台灣獼猴(*Macaca cyclopsis*)、鳳頭蒼鷹(*Accipiter trivirgatus*)及樹鵲(*Dendrocitta formosae*)最有能力攻擊位於樹冠層的鳥巢，故上述種類也可能是黑枕藍鶲重要的鳥巢天敵。另若鳥巢位置偏低或接近地面時，則可能增加許多以地面活動爲主的蛇類及哺乳類天敵，如蛇類中的南蛇(*Ptyas mucosus*)、赤背松柏根(*Oligodon formosanus*)及紅斑蛇(*Dinodon rufozonatum*)，哺乳類中的食蟹獴(*Herpestes urva*)、白鼻心(*Paguma larvata*)都是研究範圍內常見的地面巢天敵。台灣獼猴除會掠食樹冠層的鳥巢外，同時也是地面巢的重要天敵之一。此外，文獻中經常提及的重要鳥巢天敵－松鼠類(如赤腹松鼠 *Callosciurus erythraeus*) (Martin 1993; Sieving and Wilson 1998)，雖然在研究地點並不罕見，但根據陳(2007)的全時錄影研究結果，並未發現松鼠掠食研究範圍內的鳥巢。

在前述的棲地特性及天敵組成的背景之下，我們並未發現人類活動頻度、巢支撐度、巢樹類型、巢覆蓋度及水源等因子，對黑枕藍鶲鳥巢被天敵掠食的命運有顯著的影響。人類活動對鳥類天敵的影響不一，得視活動的類型、頻度及天敵的種類而定，天敵可能被阻擋

也可能增加(Filliater *et al*. 1994; Debinski and Holt 2000)。而以研究範圍的棲地特性及有限的開發程度，特別是針對黑枕藍鶲經常築巢的樹冠層而言，天敵應可自由活動。至於巢支撐度、巢樹類型、巢覆蓋度及水源等因子，固然對某些鳥類繁殖確實有影響，但一則其作用與天敵關係不明顯，如水源可能主要與食物有關(Bekoff *et al*. 1987; Atlegrim 1992)，巢支撐度則也許和惡劣天候較爲有關，二則反應黑枕藍鶲對棲地廣泛的適應幅度，如可使用的巢樹種類，或反之是顯現出其本身特性的限制或是與其他需求間的折衝(trade-off)，如對巢枝形態的要求與不隱蔽的鳥巢。另外，覆蓋度對以嗅覺及溫覺覓食的蛇類，在搜尋巢中的蛋及幼雛的阻礙不大(Schieck and Hannon 1993; Weidinger 2002)，而蛇類又是研究範圍內主要的天敵(陳 2007)，這也是巢上方的覆蓋度對黑枕藍鶲繁殖成功與否的影響不顯著的可能原因之一。

然而，在同樣的天敵組成及棲地特性之下，鳥巢是否容易接近確實會影響到黑枕藍鶲鳥巢被天敵捕食的機率。我們一開始由 2 個方向來描述鳥巢是否容易爲天敵所接近，地面天敵考量鳥巢距離地面的垂直高度及距主幹的距離，上層天敵的部分則認爲距離樹冠邊緣愈遠，愈可以降低被天敵發現及接近的機率。模式比較及 likelihood ratio test 的最終結果顯示，距離樹冠邊緣的距離愈遠，鳥巢被天敵掠食機率會下降，因此如何避免來自上層天敵的威脅，應是黑枕藍鶲築巢時的重要考量之一。不過，由研究範圍內天敵的組成分析來看，來自地面的天敵壓力並不比上層來得小，而距離地面垂直高度愈高及主幹較遠的巢，理應有利於避開哺乳類及爬蟲類的掠食，而可有較高的繁殖成功率(Nilsson 1984; Schieck and Hannon 1993; Wilson and Cooper 1998; Schmidt 1999; Kershner *et al*. 2001; Schmidt *et al*. 2001; Peterson *et al*. 2004)。關於此點，我們認爲黑枕藍鶲在選擇築巢位置時，來自地面的鳥巢天敵其實是極爲重要的選擇壓力，因爲不管繁殖成功(鳥巢高度 = 4.23m, SD = 2.52m)或被天敵掠食的鳥巢(平均高度 = 3.32m, SD = 1.64m)，以其築巢所在巢樹高度(繁殖成功巢樹平均高度 = 8.25m, SD = 4.87m；被天敵掠食鳥巢巢樹平均高度 = 5.77m, SD = 2.99m)而言(附錄)，主要都位於巢樹中層或樹冠中下層，鮮少築於非常接近地面而容易遭受地面天敵攻擊的地點。

另研究結果亦顯示巢樹高度愈高，鳥巢被天敵掠食的機率會隨之下降。原巢樹大小模式包含巢樹胸高直徑(DBH)及高度 2 因子。一般而言，樹高與 DBH 會呈顯著正比關係，但本研究中兩者關係並不高(r = 0.45)，這反應黑枕藍鶲在研究範圍內利用許多細高的巢樹，即竹類與藤蔓類。高的巢樹，除可提供較高的巢位外(巢樹高度與巢距地面垂直高度相關性係數，r = 0.84)，較厚或較寬的樹冠也可以提供較多的潛在巢位或增加天敵搜索的空間，進而使得天敵尋獲鳥巢的機率下降(Bowman and Harris 1980; Martin 1993)。

本研究結果顯示黑枕藍鶲在台灣中部丘陵以麻竹及次生闊葉樹爲主的棲地形態中，築於較高的巢樹和築於距離樹冠邊緣較遠的巢，可有效地降低被鳥巢天敵掠食的機會。鳥類的巢位是個體爲求最高適存度(fitness)下長期演化的結果。黑枕藍鶲在其整體分布範圍內，不僅可廣泛利用各種樹林性棲地類型且族群數量很普遍(Coates *et al*. 2006)，故預期在本身生物特性的限制之下，其應擁有相當的塑性(plasticity)(Ghalambor and Martin 2002)以適應不同棲地類型下的天敵壓力、食物來源和可利用的巢位棲地，建議未來相關研究可擴大研究的空間尺度，以瞭解在不同開發程度的環境下，地景(landscape)、巢位(nest patch)及鳥巢微環境等不同尺度的各種因子，對黑枕藍鶲繁殖的相對重要性，同時也可瞭解黑枕藍鶲在不同棲地形態下的應對策略。

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附錄、黑枕藍鶲(*Hypothymis azurea*)繁殖成功與被天敵掠食之巢位棲地特徵測量值(mean ± SD; 括弧內爲 median 及 ranges)

Appendix. Measurements (mean ± SD; median and ranges in parentheses) of nest-site characters for succeeded nests and depredated nests of the black-naped monarch (*Hypothymis azurea*) in Yunlin County, Taiwan, 2007-2008

Nest-site variables	Succeed nests (n = 22)	Depredated nests (n = 27)
Plant heights (m)	8.25 ± 4.87 (7, 1.4 - 20)	5.77 ± 2.99 (6, 1.3 - 12)
DBH (cm)	16.13 ± 17 (8.76, 1.8 -77.39)	10.71 ± 9.36 (8.28, 0.8 - 42.68)
Canopy covers (%)	79.39 ± 6.29 (80.6, 67.08 - 90.48)	76.75 ± 9.78 (79.04, 56.68 - 92.3)
Diameters of support branches (mm)		
Thickest twigs	7.68 ± 2.39 (6.83, 4.2 -12.15)	6.97 ± 2.06 (6.95, 3.05 - 10.6)
Thinner twigs	4.61 ± 1.57 (4.15, 2.45 - 8.25)	4.21 ± 1.71 (3.65, 1.95 - 8.85)
Means	6.14 ± 1.78 (5.54, 3.55 - 8.25)	5.59 ± 1.63 (5.78, 2.9 - 8.88)
Distances from stem (m)	1.62 ± 1.25 (1.5, 0 - 5.4)	1.17 ± 1.05 (1 , 0 - 4.5)
Nest heights (m)	4.23 ± 2.52 (4.3, 0.9 - 12)	3.32 ± 1.64 (3.4 , 1 - 6.8)
Distances of nest from foliage edge (m)	2.35 ± 1.32 (2.1, 0.6 - 5.2)	1.55 ± 0.99 (1.5, 0.2 - 4.5)
Distances to nearest road (m)	145.25 ± 270.12 (17.5, 0 - 900)	217.91 ± 612.77 (5.3, 1.5 - 2600)
Distances to nearest building (m)	290.75 ± 380.97 (90, 3 - 1500)	692.15 ± 1287.82 (250, 10 - 5000)
Distances to nearest road (m)	89.16 ± 89.96 (65, 0 - 280)	116.31 ± 112.81 (65, 0 - 420)

魚類棲地多樣性與空間層級系統之關係探討及其於溪流復育之應用

Relationship between Fish Habitat Diversity and Hierarchical Spatial Framework and Its Application in River Restoration

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摘　要

本研究於高屏溪流域藉由單位棲息地之電魚法進行魚類棲地喜好度之生態調查。結果顯示台灣間爬岩鰍、高身鯝魚等不同魚種偏好不同特性之棲地(湍瀨、深流與深潭)，即使同一魚種於生命週期不同階段所需求之棲地亦不同，如高身鯝魚幼魚偏好之底質粒徑較成魚廣泛，成魚主要生活於大漂石與小漂石棲地，成、幼魚對水深及流速之偏好也有所不同。將採樣之微棲地尺度(單位棲息地)擴大爲區段尺度(segment scale)與溪流尺度(stream scale)時，亦可發現台灣間爬岩鰍與高身鯝魚的成魚與幼魚之棲地，分別位於兩種不同物理環境型態的區段溪流，如台灣間爬岩鰍成魚在底質粒徑較大的區段溪流中有較大的魚類密度，而幼魚卻是與成魚相反。故進行魚類棲地復育設計時，不僅需要考量區段尺度的溪流棲地多樣性，溪流尺度的河川縱向棲地多樣性與棲地連結亦是必須的。

Abstract

We used electrofishing to investigate microhabitat preferences of fishes in the Kao-Ping River. The results showed that the preferences differed at the reach scale among fish species (e.g. *Hemimyzon formosanum* and *Onychostoma alticorpus*) as well as between the adult and juvenile fish. Juveniles of *O. alticorpus* had a wider range of substrate preferences, while the adults only preferred the substrates composed of small and big boulders. They also differed in water velocity and depth preferences. In the segment scale and stream scale, the adults and juveniles of *H. formosanum* and *O. alticorpus* occurred in different areas of the stream with different physical environments. The density of the adults of *H. formosanum* was higher in areas with large sediment sizes but the juveniles in areas of the small sizes. For stream habitat restoration, habitat diversity at the segment scale and different habitat preferences of adult and juvenile fish should be incorporated in the design.

關鍵詞：台灣間爬岩鰍、高身鯝魚、空間層級系統、河川縱向棲地多樣性、區段溪流棲地多樣性

Key words: fish, *Hemimyzon formosanum*, *Onychostoma alticorpus*, hierarchical spatial framework, segment scale habitat diversity

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緒　言

溪流的物理棲地型態對維持魚類族群之穩定及多樣性極爲重要，瞭解魚類族群及溪流物理棲地間之關係，並進一步考量空間與時間尺度下的層級式棲地分類(hierarchical classification)有助於了解溪流生態與河川有效管理(Ward 1989)。Frissell *et al.* (1986)利用空間層級式系統將溪流棲地予以分類，依不同之空間尺度大小，溪流棲地可分爲 5 類，由大到小分別爲溪流系統(stream system)、區段溪流系統(segment system)、河段系統(reach system)、深潭／湍瀨系統(pool/riffle system)與微棲地系統(microhabitat system)；Thomson *et al.* (2001)進一步說明區段溪流系統會因溪流的大小、級序或型態而有不同物理環境型態，例如必須注意的，雖有類似之物理棲地環境條件，在某一空間尺度被評估爲良好之棲地，在另一空間尺度卻不一定是好的棲地。Harvey *et al.* (2008)亦提出不同物理環境型態的區段溪流系統會有不同特性之棲地群集：第一種河段爲階梯式深潭棲地(step-pool)，主要是由階梯流(cascade)、湍瀨與深流(run)所組合之河相，其分布於溪流坡度較陡與高程位置較高之上游河川；第二種河段爲湍瀨／深潭棲地，主要是由湍瀨、深流與淺流(glide)所組合之河相，其分布於溪流坡

度中等與高程位置中等之中游河川；第三種河段爲淺流深潭棲地(glide-pool)，主要是由深潭、深流與淺流(glide)所組合之棲地群集，其分布於溪流坡度平緩與高程位置較低之下游河川。

水力棲地分類系統必須經由與生物資料之連結，才可提供有效的資訊於溪流棲地復育之應用(Clarke *et al*. 2003)。Schwartz and Herricks (2008)於區段溪流尺度下將棲息地分爲 9 個中型棲息地類型(mesohabitat)，討論每個棲地之特性與魚類棲地利用之關係，並指出不同之魚類所需求之棲地有所不同，區段尺度下的溪流棲地多樣性對魚類來講是重要的。孔(2006)指出因雨旱季節差異，魚類會呈現不同棲地利用之情形，雨季時台灣石賓(*Acrossocheilus paradoxus*)普遍分布在溪流各處，旱季時，小魚在急流內，中、大魚則在潭區。大嘴鱸(*Micropterus salmoides*)成魚在夏季利用溪流迴水環境，秋季時爲了適應低水位而遷移至無流速、具有豐富水生植物與較深的河岸區(Karchesky and Bennett 2004)。胡及葉(2002)利用採樣與物理棲地參數結合，建立台灣特有魚種(台灣石賓、台灣間爬岩鰍與明潭吻鰕虎)水深與流速適合度値，其中台灣間爬岩鰍適合之流速爲 0.8-1.2 m/s、水深爲 10-40 cm。以上的成果說明了以生物資料來確認棲地分類的重要性。

功能棲地(functional habitat)係採由下至上的方式來探討棲地與生物之間的關係，可分爲 16 種類型，主要分類依據是由底質類型或是水生植物類型來區分，每種功能棲地都有明顯不同的無脊椎動物群集(Harper *et al*. 1998)；水流型態物理棲地(physical biotopes)則爲由上至下的方式來探討物理棲地因子與生物間之關係，其主要分類依據爲表面水流型態或者是量化之雷諾數與福祿數(Newson *et al*. 1998)；近 10 年來，學者們將功能棲地與水流型態做連結(Newson *et al*. 1998; Harvey *et al*. 2008)，如此一來就能將生物資料(如無脊椎動物)與物理環境做連結，可提供河川管理者依不同的復育標的，選擇合適之棲地類型。然而以上之研究集中於河川物理環境因子之探討，其與魚類棲地利用之關係則較爲缺乏(Clifford *et al*. 2006)，甚至認爲幼魚的棲地偏好不固定會影響分析之結果，反而將幼魚資料剔除，不列入研究分析中(Chisholm *et al*. 1987)，但是一個良好之復育設計應考量到魚類生命週期不同階段所需求之棲地，並分別考量各階段之棲地適合度指標，才能有效地達到棲地復育之目標(Raleigh *et al*. 1986)。

建立量化溪流棲地分類系統，對於評估棲息地之狀況與溪流生態之健康非常重要(Barbour *et al*. 1999)。有關國內棲地分類系統，汪(2000)將大甲溪上游到下游的水域型態分類爲 5 種，分別爲淺流、淺瀨、深潭、深流與岸邊緩流。Vadas and Orth (1998)認爲過去研究將棲地粗分爲 3 種(如：深潭、湍瀨與深流)對河川管理目的是不夠的，因爲實際上潭是有深有淺，湍瀨亦是有慢有快，將棲地細分亦有助於了解魚類對棲地之偏好。粗略分類的系統通常缺乏量化之標準(如水深、流速與底質)，要利用視覺精確辨識不同類型之棲地是非常困難，視覺上看起來相似之兩個湍瀨，經過實際測量後其水力因子、底質粒徑與底質穩定度都明顯不同(Pedersen and Friberg 2007)。

本研究結合上述文獻之優點，並以溪流型態與棲地尺度爲核心，利用量化之數據說明魚類於生命週期不同階段其棲地利用與物理環境因子之關係，並強調溪流型態之整體性在魚類保育與復育上的重要性。

材料與方法

一、採樣地點與空間層級系統之關係

本研究爲了解不同物理環境下之棲地與魚類棲地利用之關係，故選點時以主觀的方式，選擇在視覺上物理環境型態不同之採樣站作爲

研究地點。本研究於高雄縣高屏溪支流楠梓仙溪野生動物保護區內進行魚類生態調查，採樣站爲民權大橋站(Min-Cyuan Bridge)與 12 號橋站(Twelfth Bridge)，河川級序都爲 4 級，屬於自然的溪流環境。每個採樣站中以隨機取樣方式，分多天由下游往上游採集，民權大橋站有 62 個單位棲地(1m × 1m的單位電格)，12 號橋站有 42 個單位棲地。

圖 1. 楠梓仙溪採樣點(使用網際水利地理資訊系統 http://gmap.wra.gov.tw/wrahub_3wgis/)。

Fig. 1. The sampling sites at the Nan-Zih-Sian River (From http://gmap.wra.gov.tw/wrahub_3wgis/).

表 1. 採樣站之 TWD97 二度分帶座標及物理環境資料

Table 1. TWD97 coordinates and physical environmental data of sampling sites at the Nan-Zih-Sian River

Environmental data	Min-Cyuan Bridge #1	Min-Cyuan Bridge #2	Twelfth Bridge #1	Twelfth Bridge #2
X coordinates	218678	218679	215906	215996
Y coordinates	2571705	2571889	2566768	2566842
Gradient	0.0593	0.0437	0.0211	0.0111
Sampling range (m)	80	90	60	110
Average width of water surface (m)	27	28	33	34

本研究採樣時以微棲地尺度來收集魚類資料，接著統計分析魚類棲地利用與量化物理棲地因子之關係；最後，利用不同單位棲息地(單位電格)之結合，將微棲地尺度擴大爲區段尺度與溪流尺度，以找尋較大尺度棲地因子與成、幼魚棲地利用之關係；而非利用河段或區段溪流系統的變數(如Fayram and Mitro 2008)。民權大橋站與 12 號橋站都可分爲兩個小採樣站，各兩小採樣站都在橋之上游(圖 1)。每個小採樣站都包含多種不同類型之棲地，如深潭、湍瀨、淺流、深流等，故民權大橋 1 號站、民權大橋 2 號站、12 號橋 1 號站與 12 號橋 2 號站都可說是河段尺度下之棲地，表 1 所示爲各小站之座標與物理環境相關資料。若以民權大橋站與 12 號橋站比較的話，溪流坡度有漸漸變緩的趨勢。將民權大橋 1 號站與民權大橋 2 號站(或 12 號橋 1 號站與 12 號橋 2 號站)合併時，兩站之間並無支流流入，且兩站之距離約 200m 長，符合區段尺度之空間長度，民權大橋 1 號站與民權大橋 2 號站合併後稱之爲民權大橋站，12 號橋 1 號站與 12 號橋 2 號站合併後稱之爲 12 號橋站，故民權大橋站與 12 號橋站可算是區段尺度下之棲地。民權大橋與 12 號橋兩站距離約 8 km (直線距離約 5.5 km)，已符合溪流尺度之空間長度，故本研究在楠梓仙溪當民權大橋與 12 號橋資料合併時亦可算是溪流尺度的棲地研究，詳細情形請參考圖 2。

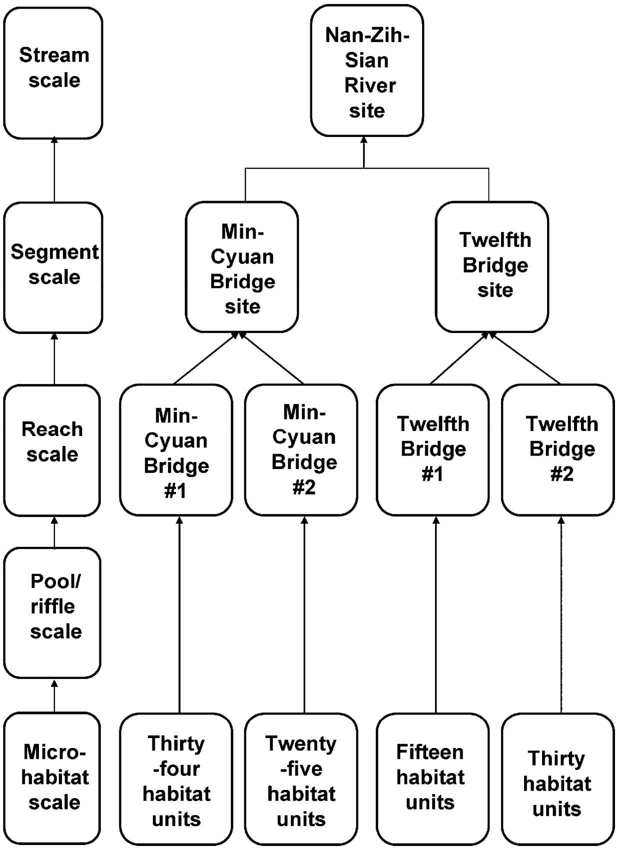

圖 2. 空間層級系統與棲地之關係。

Fig. 2. A hierarchical spatial framework of fish habitat.

二、物理棲地之量測方式

本研究所使用之電棒長度爲 1m，兩電棒距離 1m，形成 1m × 1m 的電格範圍；水深與流速利用流速計量測，量測點爲均勻分布於電格中，分別各量 9 點，並取其平均值爲該電格之平均水深與平均流速；底質方面採用 Platts *et al.* (1983)的表面目視法(surface-visual-method)，配合本研究自製之底質粒徑百分板來輔助測量電格內之底質粒徑百分比；底質粒徑的分類則參考Bovee and Milhous (1978)所分類之方式，但作者將底質分類爲 9 類，並給予底質粒徑序號 1-9，由於分得太細對於粒徑計算百分比時會增加許多工作時間與分析複雜度，亦有研究者建議將鄰近範圍底質粒徑合併(Knighton 1998; 汪 1990)，故本研究將大、小鵝卵石合併爲鵝卵石(cobble)，大、小礫石合併爲礫石(gravel)，砂以下合併爲細顆粒(sand)，底質序號分別改爲 5.5、3.5 與 1.5，再加上小漂石(small boulder)與大漂石(big boulder)共計 5 類，詳細底質分類與序號如表 2 所示。計算底質平均粒徑方面，Statzner *et al.* (1998)的計算方法爲 $(5C_1 + 3C_2 + C_3)/9$，其中 C_1、C_2 與 C_3 爲電格內出現頻率最高的前三名底質序號。本實驗因已有計算電格內各種粒徑所含的百分比，故參考 Statzner *et al.* (1998)的計算方法，並將它修改如下式：

底質平均粒徑序號(單位電格m^2) $= \alpha_1 * 1.5 + \alpha_2 * 3.5 + \alpha_3 * 5.5 + \alpha_4 * 7 + \alpha_5 * 8$

其中α_1、α_2、α_3、α_4 與α_5 分別爲細顆粒、礫石、鵝卵石、小漂石與大漂石在電格的百分比。

表 2. 粒徑分類比較表

Table 2. The classification of substrate sizes

	Bovee and Milhous (1978)		This study	
Substrate size (mm)	Substrate type	Code	Substrate type	Code
<1	Silt	1	Sand	1.5
1-2	Coarse sand	2		
2-16	Fine gravel	3	Gravel	3.5
16-64	Coarse gravel	4		
64-128	Pebble	5	Cobble	5.5
128-256	Cobble	6		
256-512	Small boulder	7	Small boulder	7
>512	Big boulder	8	Big boulder	8
	Rock	9		

三、統計方法

本研究所使用的統計工具爲多變量變異數分析(multivariate analysis of variance)與獨立性卡方檢定(Chi-square test)。多變量變異數分析可利用來客觀地判別不同採樣站的棲地因子是否顯著差異；獨立性卡方檢定則可利用來判別成、幼魚的數量分布在不同採樣站中是否有顯著差異。

結　果

一、魚類採樣結果

本研究採樣時間爲 2008 年 10 月至 2009 年 4 月，共計採集 3 科 5 種 945 隻魚，其中優勢魚種爲台灣間爬岩鰍(*Hemimyzon formosanum*)計 775 隻、高身鯝魚(*Onychostoma alticorpus*)計 242 隻與明潭吻鰕虎(*Rhinogobius candidianus*)

計 355 隻，次要魚種則爲台灣石鱝(*Acrossocheilus paradoxus*)與台灣馬口魚(*Candidia barbata*)。爲了解魚類於生命週期不同階段所偏好棲地是否不同，參考 Raleigh *et al*. (1986)的方式利用體長將魚類分爲成魚與幼魚，由於魚類體長的伸展與年齡的增加有比例關係，由此，通常以體長做爲年齡推定的依據未必是不可行的(朱 2001)。因成、幼魚之體長各專家說法不一，本研究以參考文獻與詢問專家(曾晴賢，私人通訊)方式，來訂定成、幼魚之體長界線(表 3)，爲了降低成、幼魚的誤判率，本研究不以二分法的方式來區分，而將中間某一範圍之體長不列入資料分析，以增加成、幼魚之判定可靠度。

表 3. 台灣間爬岩鰍及高身鯝魚之成魚與幼魚之分類方式

Table 3. The classification of adult and juvenile fish by body length for *Hemimyzon formosanum* and *Onychostoma alticorpus*

Species	This study	陳及方(1999)	方等人(1996)	曾晴賢(私人通訊)
H. formosanum	Adults (> 5 cm) Juveniles (< 4 cm)	Sexual maturity (4-8 cm)		Adults (> 5 cm)
O. alticorpus	Adults (> 15 cm) Juveniles (< 8 cm)	Sexual maturity (20-30 cm)	Juveniles (< 8 cm)	Adults (>15 cm)

二、採樣站的物理環境因子比較

由多變量變異數分析結果得知(表 4)，民權大橋站與 12 號橋站流速與水深相近($p > 0.05$)，但底質粒徑方面卻有顯著差異($p < 0.05$)。由於河床自然演變的結果，民權大橋與 12 號橋的溪流型態已漸漸產生不同，如圖 3 與圖 4 所示，民權大橋無明顯主(水深最深連線)、副河道，水面寬較小、蜿蜒度低；12 號橋則相反。民權大橋站主要的底質爲大、小漂石；12 號橋主河道多由大、小漂石與細顆粒組成(水面寬約 7m)，副河道則由小漂石與鵝卵石底質所組成(水面寬約 27m)。

表 4. 民權大橋與 12 號橋溪流型態差異之統計結果

Table 4. The results of MANOVA for the differences in physical stream types of the Nan-Zih-Sian River between Min-Cyuan Bridge and Twelfth Bridge

Environmental variables (average)	Min-Cyuan Bridge	Twelfth Bridge	Difference	*P-value*
Substrate (code)	6.84	5.95	0.89	0.000
Velocity (m/s)	0.794	0.721	0.073	0.834
Depth (cm)	38	37	1	0.995

三、魚類於不同採樣站間之分布

本研究進行獨立性卡方檢定時，發現有較多台灣間爬岩鰍與高身鯝魚成魚出現於民權大橋站，較少出現於 12 號橋站；相反的，台灣間爬岩鰍與高身鯝魚幼魚較多出現於 12 號橋站，較少出現於民權大橋站(圖 5，圖 6)；台灣間爬岩鰍與高身鯝魚的成、幼魚密度於民權大橋站與 12 號橋站的分布型態差異相當懸殊($p < 0.05$)，12 號橋站之台灣間爬岩鰍與高身鯝魚幼魚密度幾乎是民權大橋站的 3 倍(表 5)。這也表示了台灣間爬岩鰍與高身鯝魚成魚是較偏好民權大橋站所具備的物理環境型態，而幼

魚則較偏好 12 號橋站所具備的物理環境型態。

圖 3. 民權大橋以大、小漂石爲主且無明顯主副河道的溪流型態。

Fig. 3. The stream type of big and small boulders with no obvious main channel at Min-Cyuan Bridge.

圖 4. 12 號橋底質粒徑廣泛且有明顯主副河道之溪流型態。

Fig. 4. The stream type of wide range substrates with obvious main channel at Twelfth Bridge.

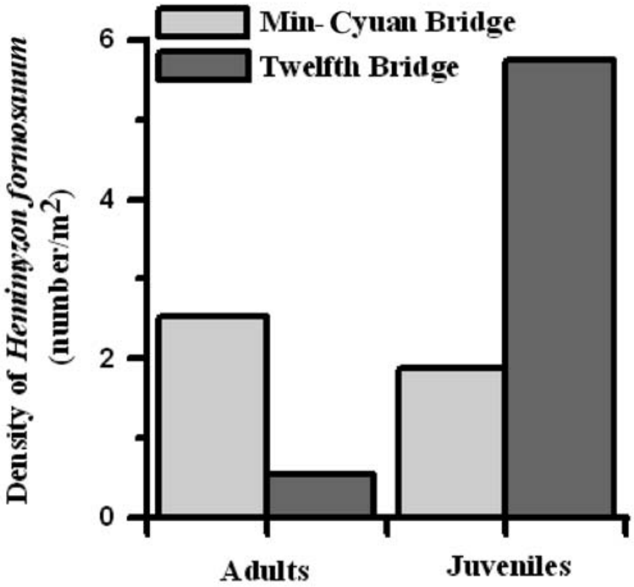

圖 5. 台灣間爬岩鰍於民權大橋站與 12 號橋站之分布比較。

Fig. 5. The density distributions of *Hemimyzon formosanum* in the Nan-Zih-Sian River at Min-Cyuan Bridge and Twelfth Bridge.

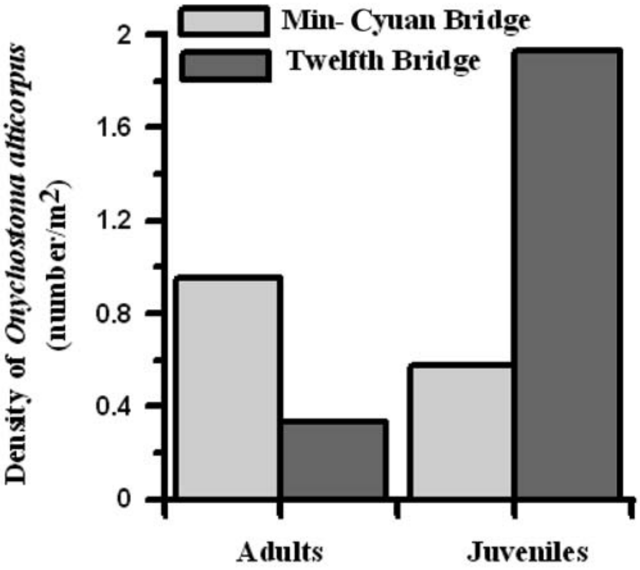

圖 6. 高身鯝魚於民權大橋站與 12 號橋站之分布比較。

Fig. 6. The density distributions of *Onychostoma alticorpus* in the Nan-Zih-Sian River at Min-Cyuan Bridge and Twelfth Bridge.

表 5. 台灣間爬岩鰍與高身鯝魚之成、幼魚於民權大橋與 12 號橋之數量分布統計結果

Table 5. A comparison of adult and juvenile density distributions in the Nan-Zih-Sian River between Min-Cyuan Bridge and Twelfth Bridge for *Hemimyzon formosanum* and *Onychostoma alticorpus*

Species	Method	Chi-square value	*P*-value
H. formosanum	Pearson Chi-square	30.26	0.000
	Phi (Φ)	0.543	0.000
O. alticorpus	Pearson Chi-square	13.68	0.001
	Phi (Φ)	0.540	0.001

討論

在了解魚類棲地需求與棲地型態上，本文所探討的空間尺度，主要集中於區段系統與溪流系統。爲有助於了解在不同空間尺度下，魚類棲地需求與棲地型態之關係，並說明爲何台灣間爬岩鰍與高身鯝魚成、幼魚於民權大橋與12 號橋的數量分布有顯著差異，以下就台灣間爬岩鰍與高身鯝魚成、幼魚對底質粒徑及水深、流速之偏好，探討魚類棲地需求與棲地型態之關係。

台灣間爬岩鰍成、幼魚偏好棲地之水深與流速差異不大，在流速範圍於 0.75-1.8 m/s 與水深於 52 cm以下，對台灣間爬岩鰍成、幼魚是一個適合生存之棲地(呂 2009)。台灣間爬岩鰍可適應的流速比大部分魚類快，推測可能的原因爲其體型呈扁平且具吸盤。方等人(1995)亦指在水流流速高情況下，容易對魚類產生紊流效應與遮蔽性效應而不容易掌握獵物之行蹤，進而減少被捕食之機率。比較不同的是，台灣間爬岩鰍成、幼魚對底質粒徑之偏好有顯著差異，成魚偏好大漂石，而幼魚偏好鵝卵石(表 6，圖 7)。

高身鯝魚成、幼魚都可適應於流速 1.05 m/s 以下之棲地；流速超過 1.05 m/s 之後，高身鯝魚幼魚就無法適應，但成魚可適應於流速大於 1.05 m/s 之棲地；高身鯝魚成魚偏好之水深與底質粒徑值亦大於幼魚，高身鯝魚成魚生活範圍較集中於大粒徑(平均序號 6.49-8.0，大、小漂石) (表 6 與圖 8)，其原因爲大、小漂石之較大粒徑的底質有利於附著性藻類生存，且高身鯝魚主要食物來源爲附著性藻類(方等人 1995)，Wang (1989)亦指出鯝魚族群的靜態生產量與溪流中之巨石底質百分比組成呈現正相關，與砂土百分比組成呈現負相關；幼魚的生活範圍則較廣，大、小粒徑都可適應(序號 4.88-8.0，鵝卵石-大漂石)；水深部分，因成魚不適合生存於水深 5-22 cm 棲地(呂 2009)，其原因爲成魚體型較大，在淺水地方移動容易受限制，被鳥類捕食機率增加許多(Khan *et al*. 2004)，故偏好水深平均值較幼魚大。

最後，將水深、流速與底質合併以了解魚類棲地利用與棲地型態之關係；台灣間爬岩鰍幼魚偏好鵝卵石棲地(湍瀨、急湍瀨與深湍瀨)與底質爲複合型(鵝卵石與小漂石爲主)之湍瀨；成魚偏好大漂石棲地(湍瀨、急湍瀨與深急湍瀨)與小漂石棲地(湍瀨、深湍瀨、急湍瀨)，成、幼魚偏好之棲地大都爲湍瀨類型。高身鯝魚幼魚偏好湍瀨(鵝卵石、複合型)、深流(小漂石、複合型，礫石與大漂石之結合底質)、中等深潭(小漂石、大漂石)與深潭(小漂石，礫石與大漂石之結合底質)；成魚偏好大漂石棲地(深流、深湍瀨、深急湍瀨與深潭)、小漂石深流、複合型深潭等棲地類型，成魚與幼魚都能適應湍瀨、深潭與深流類型棲地，表示高身鯝魚對棲地偏好是廣適型魚種，本研究棲地分類之量化標準如表 7 所示。

表 6. 台灣間爬岩鰍與高身鯝魚對底質偏好之統計驗證

Table 6. The statistic tests of substrate preference of *Hemimyzon formosanum* and *Onychostoma alticorpus* in the Nan-Zih-Sian River

Species	F test	Substrate group	Substrate group	Difference in means of two substrate groups (number/m^2)	Post Hoc test (*P-value*)
H. formosanum (adults)	F_3 = (4.709, 0.000)	Big boulder	Cobble	2.74	0.016
			Cobble and small boulder	1.86	0.204
			Small boulder	1.45	0.566
H. formosanum (juveniles)	F_3 = (8.184, 0.004)	Cobble	Cobble and small boulder	3.81	0.188
			Small boulder	5.43	0.007
			Big boulder	5.69	0.004
O. alticorpus (adults)	F_3 = (9.710, 0.000)	Big boulder	Cobble	1.63	0.002
			Cobble and small boulder	1.64	0.001
			Small boulder	1.05	0.321
O. alticorpus (juveniles)	F_3 = (0.850, 0.566)	Cobble	Cobble and small boulder	0.34	1.000
			Small boulder	0.16	0.824
			Big boulder	0.92	0.811

表 7. 依據水力因子分類之棲地類型

Table 7. Habitat classification based on hydraulic conditions

Velocity (m/s) Depth (cm)	Slow velocity (< 0.45)	Medium velocity (0.45-0.74)	Fast velocity (0.75-1.04)	Very fast velocity (1.05-1.8)
Very shallow depth (5-21)	Shallow pool	Riffle	Riffle	Fast riffle
Shallow depth (22-31)	Shallow pool	Riffle	Riffle	Fast riffle
Medium depth (32-51)	Medium pool	Run	Deep riffle	Deep-fast riffle
Deep depth (52-120)	Deep pool	Run	Run	Fast run

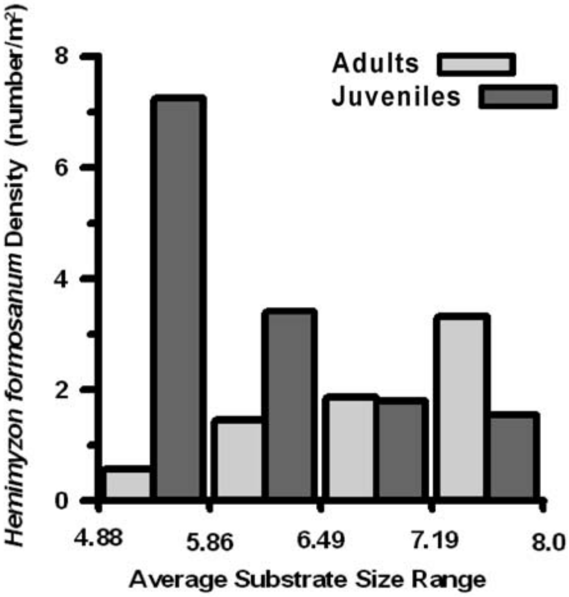

圖 7. 台灣間爬岩鰍對平均粒徑喜好度的分布型態。

Fig. 7. The density distributions of adults and juveniles of *Hemimyzon formosanum* for different substrate groups in the Nan-Zih-Sian River.

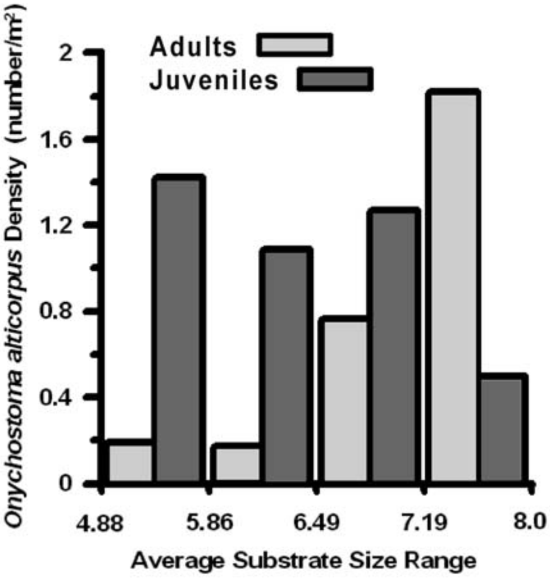

圖 8. 高身鯝魚對平均粒徑喜好度的分布型態。

Fig. 8. The density distributions of adults and juveniles of *Onychostoma alticorpus* for different substrate groups in the Nan-Zih-Sian River.

民權大橋站附近可提供較多的台灣間爬岩鰍與高身鯝魚成魚所需求之棲地，12 號橋站附近則可提供較多幼魚所需求的環境，故造成台灣間爬岩鰍與高身鯝魚之成、幼魚在民權大橋與 12 號橋分布有明顯之差異。除了成、幼魚對物理棲地偏好有不同外，其內在原因可能爲成、幼魚之體型、游泳與機動能力，或者是棲地分離策略(減少食物競爭)所造成。陳(2007)將台灣間爬岩鰍與埔里中華爬岩鰍定義爲洄游性魚種，並提到兩魚種都有向上洄游到底質粒徑較大的地方生存之習性，符合本研究成魚分布偏上游之情形。殷(1998)亦指出洄游是魚類獲得延續、擴散與增長的重要行爲特性，洄游是魚類對環境的一種長期適應，魚類透過洄游變換棲地場所，擴大對環境空間之利用，提高種族存活、攝食、繁殖與避開不良條件的能力。

當把空間尺度放在區段尺度溪流時，我們將民權大橋站與 12 號橋站的魚類棲地利用情形分開來討論。民權大橋站與 12 號橋站都採樣到相同的 5 種魚類，其中，台灣間爬岩鰍與高身鯝魚需求之棲地，已涵蓋了水力棲地多樣性的涵義一由瀨型、深流型與潭型棲地所組合的溪流。所以，棲地復育時，每個區段尺度的溪流必須注意區段溪流的水力棲地多樣性。如區段溪流中必須要有湍瀨、急湍瀨、深流、深湍瀨、深急湍瀨、岸邊緩流、中等深潭與深潭等各種組合，如此方可滿足不同魚類的棲地需求。然而，由本研究結果得知，成、幼魚分別位於不同溪流型態的區段尺度溪流中，區段尺度之水力棲地多樣性只能滿足成魚或幼魚其中之一的棲地利用，所以，必須將民權大橋與 12 號橋兩個有不同溪流型態之溪流予以連結，亦即棲地復育必須考量更大尺度(如溪流尺度)與魚類棲地利用之關係。

當空間尺度放大到溪流尺度時，在兩個區段溪流中，可能因河床底質搬運作用，產生以不同底質爲主的棲地，這兩種不同溪流型態的棲地連結，在本文中稱之爲「河川縱向棲地多樣性(底質多樣性)」。區段尺度的溪流除了原有的湍瀨、急湍瀨、深流、深潭與淺潭等形成的水力棲地多樣性，也必須考量到溪流尺度或更大尺度的河川縱向棲地多樣性。如以本研究兩魚種來說明，台灣間爬岩鰍成魚偏好大漂石急湍瀨(民權大橋站) (參考圖 9)，幼魚偏好鵝卵石急湍瀨(12 號橋站) (參考圖 10)，兩者棲地類型依水深與流速都可歸類爲急湍瀨，但因成、幼魚對底質之偏好不同，以致於棲地復育時必須考量到以不同底質爲主之急湍瀨；又如高身鯝魚成魚偏好由大漂石或小漂石組成之深潭、深流、湍瀨、急湍瀨等(民權大橋站) (參考圖 11)；幼魚偏好的底質廣泛(12 號橋站)，從以大、小漂石與細顆粒爲主的主河道到以複合型與鵝卵石爲主之副河道都是幼魚可適應之棲地(參考圖 12)。

水深、流速與底質對魚類棲地利用之影響是環環相扣的，故同時考量區段溪流的棲地多樣性與河川縱向棲地多樣性是能讓棲地復育更爲完整，因它不只能滿足生物多樣性，亦能滿足魚類生命週期不同階段所需求之棲地。簡單來說，小尺度(河段或區段尺度)的溪流棲地多樣性與大尺度(溪流或更大尺度)的溪流棲地多樣性都是棲地復育的重要考量要點，完整的河川管理政策必須能評估不同尺度下魚類棲地使用與物理環境因子之關係(Thomson *et al.* 2001; Harvey *et al*. 2008)，故本研究建議棲地復育與評估時，於最小的微棲地尺度下，需考量魚類需求之水深、流速、底質等等；於深潭湍瀨尺度下，需考量魚類所偏好之棲地類型(如深潭、湍瀨、深流等)；於河段或區段尺度下，需考量河段或區段溪流的水力棲地多樣性與棲地之組成(棲地群集)，來滿足各魚種之棲地需求；於溪流尺度下，需考量河川縱向棲地多樣性，來滿足魚類於生命週期不同階段之棲地需求或者是魚類洄游以延續、擴散與增長族群；最後，對於河川縱向棲地多樣性之復育，亦應依循底質粒徑從上游到下游慢慢變小之自然特性

(Church 2002)，符合自然之復育工程可減少失敗之機率。

圖 9. 民權大橋之大漂石急湍瀨。

Fig. 9. A big boulder-fast flowing riffle of the Nan-Zih-Sian River at Min-Cyuan Bridge.

圖 10. 12 號橋之鵝卵石急湍瀨。

Fig. 10. A cobble-fast flowing riffle of the Nan-Zih-Sian River at Twelfth Bridge.

圖 11. 以大、小漂石組成之各類型棲地(民權大橋)。

Fig. 11. A habitat assemblage composed of big and small boulders in the Nan-Zih-Sian River at Min-Cyuan Bridge.

圖 12. 鵝卵石-大漂石組成之各類型棲地(12 號橋)。

Fig. 12. A habitat assemblage composed of substrates with cobbles to big boulders in the Nan-Zih-Sian River at Twelfth Bridge.

結論

本研究採用於視覺上棲地物理環境型態有所不同的採樣站做比較，民權大橋站與 12 號橋站之底質粒徑在統計上有顯著差異，民權大橋站底質粒徑以大漂石與小漂石爲主，水面平均寬約 28m；12 號橋站副河道底質粒徑以複合型與鵝卵石爲主，水面寬約 27m，主河道較常出現大漂石、小漂石與細顆粒，水面寬約 7m；而兩站之流速與水深平均值在統計上皆無顯著差異。

台灣間爬岩鰍與高身鯝魚可能因成、幼魚之體型、游泳與機動能力、洄游習性或者是棲地分離策略等因素，導致一、在民權大橋站與 12 號橋站的成、幼魚數量分布有顯著差異；二、棲地偏好或生態區位不同。台灣間爬岩鰍與高身鯝魚幼魚在 12 號橋站之魚類密度爲民權大橋站的 3 倍左右。

棲地復育與評估時，應考量以下幾要點：一、最小的微棲地尺度－考量魚類需求之水深、流速與底質；二、深潭湍瀨尺度－考量魚類偏好之棲地類型(如深潭、湍瀨、深流等)；三、河段或區段尺度－考量河段或區段溪流的棲地多樣性與棲地之組成，來滿足各魚種之棲地需求；四、溪流尺度－考量河川縱向棲地多樣性，來滿足魚類於生命週期不同階段之棲地需求，或者是滿足魚類洄游以延續、擴散與增長族群；五、對於河川縱向棲地多樣性之復育，應考量底質粒徑的分布從上游到下游逐漸變小，符合自然之復育工程可減少失敗之機率。

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清潔劑應用在動物 DNA 遺傳物質的保存

Laundry Detergents as Animal Tissue Preservatives for DNA Analyses

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摘　要

本文測試保存在常溫下酒精中 2 個月到 9 年間的動物組織樣本的 DNA 衰退程度，以及幾種清潔劑在長時間、高溫環境下保存DNA組織樣本的效果，並討論酒精在現今動物DNA組織樣本保存上的優缺點，及其應用在 DNA 序列判讀等分子生物學研究技術上的限制。測試結果顯示，保存在 95%酒精中的組織樣本其DNA 結構在 7 年後會有明顯的衰退現象，以 70%酒精保存 DNA 的效果亦相當差。而多種清潔劑在一定的時間內對於 DNA 物質的保存效果與 99.5%的無水酒精相近，但並非所有的家用清潔劑都適用於 DNA 樣本保存。

Abstract

Several brands of laundry detergents were tested as the preservatives for animal tissues in DNA analyses. Their DNA degradations were compared to those from the tissues preserved in ethanol and DNA preservation buffer, and from cryopreservation (immersion in liquid nitrogen). The results showed that the tissues preserved in 99.5% ethanol and DNA preservation buffer were as good as those from cryopreservation, but those in 70% ethanol degraded faster. Some (not all) of the detergents brands tested were found to be as good as those preserved in concentrated ethanol and DNA preservation buffer. Preservation in a detergent-based solution is an easy and economic way for tissue preservation in DNA analyses.

關鍵詞：清潔劑、動物組織樣本、DNA 保存、DNA 衰退

Key words: detergents, animal tissue specimen, DNA preservation, DNA degradation

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緒 言

近年來，由於各項分子生物學操作技術的進步，技術門檻大幅降低，DNA 序列分析已經成爲分類學、演化生物學、族群遺傳學及生物地理學等各領域研究應用的利器之一。對於數量稀少的珍稀或瀕危物種，分子生物技術可以僅就少量的血液或組織樣本，如一小片魚鰭或是蜥蜴自割的尾巴，在不嚴重危害研究對象的情形下取得大量的遺傳資訊，對其族群的傷害可減至最低。也隨著實驗步驟的精簡及單項操作所需費用日益降低，一般應用 DNA 定序技術的生物學研究已經很難滿足於少量樣本數的分析結果。然而，大量的分析樣本往往需要長時間的蒐集，衍生出來的樣本保存問題則隨著數量及保存時間的增加而逐漸浮現。

在一般博物館的動物標本蒐藏中，除了獸類及鳥類常用皮毛剝製法製作標本外，體型較小的兩爬動物、魚類及昆蟲以外的無脊椎動物大多以浸液標本的形式保存。常見的浸液標本主要可分爲福馬林(formalin)固定及酒精直接浸漬兩種製作方法。福馬林爲 40%的甲醛(formaldehyde)稀釋溶液(Fox *et al*. 1985)。甲醛於 1859 年首次被配製後，福馬林很快地就被應用在生物標本保存技術上面(Fox *et al*. 1985; Schander and Halanych 2003)。福馬林能防止樣本腐壞，並藉由形成蛋白質結構間的鏈結結構(cross-linkages)固定生物標本的形狀，使其形態免於收縮(shrinkage)或扭曲(distortion)等變形(Fox *et al*. 1985)。然而，福馬林可能有破壞所保存樣本的DNA的副作用(Shibata 1994)。一般博物館所保存的動物浸液標本大多先以福馬林固定形態，再轉移至酒精溶液中保存，這樣的處理方式相當不利於 DNA 分析、定序等分子生物學研究(France and Kocher 1996)，很難從福馬林固定過的樣本中萃取出足量、質優的DNA來進行聚合酶連鎖反應(PCR, polymerase chains reaction)放大等實驗室操作。福馬林溶液如何影響樣本的 DNA 結構目前並不清楚(Chaw *et al*. 1980; Chang and Loew 1994; Schander and Halanych 2003)。有多篇研究報告敘述如何萃取福馬林固定過的動物標本的DNA (France and Kocher 1996; Chatigny 2000; 徐等 2002; Wandeler *et al*. 2007)，多數結果未臻理想，一般只能增幅出小於 400-500 bp 的小片段序列(Shibata 1994; Wandeler *et al*. 2007)。由於這項技術並非本文的主題，在此不多做討論。

超低溫冷凍法(cryopreservation)是已知最

佳的組織樣本保存技術(Zhang and Hewett 1998)。新鮮的組織樣本先以液態氮或乾冰處理，再以-70°C 以下的低溫保存，如此處理的樣本可以應用於大部分的分子生物學研究中。然而，除了在野外工作時很難將樣本如此處理外，超低溫設備的維護也是相當昂貴，若要全面應用於所有組織樣本的保存則需要有相當龐大的預算長期支持才行。Dessaure *et al*. (1990)提出多種可在野外工作時短時間保存動物血液或組織樣本的溶液，如酒精、抗凝血劑保存溶液(anticoagulant preservative solution) (Arctander 1988)、含十二烷基硫酸鈉(SDS, sodium dodecyl sulfate)的溶液，或是過飽和的食鹽溶液，每種溶液都有一定的效果。

以高濃度的酒精保存欲進行 DNA 萃取的血液或組織樣本，是目前許多實驗室普遍採用的方法。酒精有容易取得、容易稀釋及無毒性等優點，雖爲易燃物質，在野外或實驗室操作時只要注意火源或避免高熱環境即可。酒精樣本應保存於 4-8°C的環境中；保存於 4°C環境的酒精組織樣本會比保存於室溫或-20°C 以下的樣本更易萃取出質優的 DNA (Tegelström 1989; Zhang and Hewett 1998)。在筆者個人的經驗中，經過酒精短時間浸泡的樣本，其所萃取出的 crude DNA 在 PCR 技術應用上有時甚至較冷凍或未經處理的新鮮樣本爲佳。但是，酒精長時間保存的組織樣本依舊會有無法萃取出足夠的DNA以進行PCR增幅程序的問題存在，且保存時間越長久的樣本其萃取出的DNA質量越差。Reiss *et al*. (1995)發現甲蟲 *Amara glacialis* (Mannerhrim)保存在 70%的酒精中約 6 週後其DNA質量就會明顯衰退(degradation)。類似的衰退現象也出現在酒精保存的蜘蛛(Hormiga *et al*. 2003; Vink and Paterson 2003)等其他無脊椎動物樣本中；Vink *et al*. (2005)甚至認爲室溫下保存於 95%酒精中的蜘蛛樣本 5 天後其 DNA 就開始衰退。關於酒精保存技術對於脊椎動物 DNA 影響方面的研究則比較少。筆者由實驗室操作經驗中發現，長時間保存於酒精的組織樣本普遍存在 DNA 衰退的現象，而常溫下浸漬於酒精溶液達 10 年以上的標本所萃取的 DNA 往往濃度很低，難以進行 PCR 增幅。如 1998 年國立自然科學博物館(簡稱科博館)同仁赴越南採集的一批兩棲類酒精浸漬標本，在室溫下直接浸漬、保存於濃度 75%的酒精中，形態保存相當完好；但是在 2008 年時因研究需求取出該批標本部分大頭蛙(*Limnonectes* sp.)樣本的腿部肌肉進行DNA萃取，卻完全無法取得可供 PCR 增幅的 crude DNA。

由於以酒精保存樣本 DNA 的缺點逐漸浮現，多種非酒精的 DNA 組織樣本保存方法被發展出來，取代原本的酒精保存方式(Arctander 1988; Dessaure *et al*. 1990; Kuch *et al*. 1999)。TODD’s DNA buffer (附錄)即爲一簡易、穩定的 DNA 保存溶液。其配方以 SDS 爲主，而SDS本身即是洗滌用的清潔劑的主要成分。有趣的是，一般家庭用洗衣清潔劑就可以用來保存 DNA 組織樣本(Kuch *et al*. 1999)，有著和TODD’s DNA buffer 類似的效果。爲了加強去污能力，一般市售的家用洗衣粉都會添加能夠分解有機物質的去污劑、酵素(如 proteases 及 lipases)以及類似 EDTA 的螯化物(Kuch *et al*. 1999)，其成分恰如一般商業DNA萃取試劑組用來分離 DNA 的萃取溶液(extraction buffer) (Bahl and Pfenning 1996; Kuch *et al*. 1999)。Kuch *et al*. (1999)測試了洗滌用清潔劑用在保存兩爬動物的血液及肌肉組織樣本，以及取代慣用的DNA萃取試劑組中digest buffer的可行性，兩者都有不錯的效果。

本研究分兩個部分進行：第一部分爲利用科博館動物學組兩爬實驗室所保存的多組拉都希氏赤蛙(*Sylvirana latouchii*)酒精組織樣本，測試不同浸泡時間的樣本其 DNA 質量是否有差異，以證實長時間保存於酒精中的組織樣本會造成DNA衰退；另外，爲確定Kuch *et al*. (1999)的結論能否套用在國產的清潔劑上，並應用於

國內學者的野外工作及動物組織樣本保存策略，本研究第二部分將依循 Kuch *et al*. (1999)的測試方法並稍做修改，測試 TODD’s DNA buffer 及台灣市面上可見的幾種洗衣粉及清潔劑應用在保存組織樣本的可行性，並以相近的測試條件測試兩種酒精濃度所保存的組織樣本對照之。

材料與方法

一、酒精組織樣本 DNA 衰退與保存時間的關連性測試

選取在 6 個時間點常溫下浸漬於濃度 95% 以上的酒精中的 12 組拉都希氏赤蛙樣本(詳見表 1)同時進行萃取，萃取操作時間爲 2009/4/21，每組以 5 mg (± 0.8 mg)的新鮮組織樣本定量操作。所有樣本利用商業試劑組 Tissue and Cell Genomic DNA Purification Kit (Hopegen Biotechnology Development Enterprises)進行 DNA 萃取，操作步驟依據試劑組使用手冊，並修改 56°C 水浴時間，由手冊建議的 4 hr 延長至 16 hr。保存於酒精中的組織樣本在此步驟操作前先去掉酒精，再以眞空乾燥機(CVE-200D, EYEL4)常溫下眞空處理 10 min，確保樣本內的酒精完全去除。萃取出的 crude DNA 產物溶於 50 μl 的二次去離子水中，進行電泳時各組取出 5 μl 的 crude DNA 溶液，加上 1 μl 的 6X 染色染劑混合均勻後在 1.2%電泳凝膠中以 100V 電壓進行電泳 30 min，以估算各組樣本的相對濃度。DNA Integrated Density Value (IDV)值爲估算電泳圖上約 20 kb 分子量大小的 DNA 濃度所得的結果，顯示各組樣本占所有樣本總濃度的比例，並以最後兩組樣本(保存 2 個月)的 IDV 平均值爲分母，計算各時間點的樣本平均值相對於保存 2 個月的樣本之 DNA 濃度比值。凝膠製作時添加 HealthView Nucleic Acid Stain (Genomics)進行內染。進行電泳時同時以 1 kb DNA ladder 標定 DNA 分子量大小。電泳測試結果在波長 312 nm 的紫外光下判讀，並且利用數位照相系統(Alpha Innotech Corp.)照相保存，以 AlphaImager IS-2200 (NatureGene Corp.)影像處理軟體估算 DNA 相對濃度。

表 1. 測試在不同保存時間下 DNA 衰退情形所使用的組織樣本資訊，包括國立自然科學博物館(NMNS)標本號、標本採集時間以及樣本保存時間(所有樣本皆爲拉都希氏赤蛙 *Sylvirana latouchii* 的腿部肌肉)

Table 1. Data on the *Sylvirana latouchii* specimens used in the DNA degradation experiment

	NMNS[1] catalogue numbers	Collection dates	Preservation period (month)	DNA Integrated Density Value[2] (%)	Crude DNA[3] (%)
1	#16445	2000.05.01	107	1.50	10.49
2	#16446			1.50	
3	#16529	2002.02.12	86	3.70	32.52
4	#16530			5.60	
5	#12995	2004.03.27	59	16.50	89.51
6	#12996			9.10	
7	#16155	2006.07.18	34	9.50	60.49
8	#16156			7.80	
9	#17122	2008.04.01	12	6.30	56.99
10	#17123			10	
11	#17754	2009.02.14	2	14.30	-
12	#17755			14.30	

[1] National Museum of Natural Science, Taiwan.

[2] Estimated DNA concentrations in molecular weight of about 20 kb.

[3] Proportion of DNA concentration equivalent to that at 2-month preservation.

二、清潔劑保存 DNA 物質的適用性測試

本研究測試了5種市售的洗衣粉、洗衣精以及洗碗精，其品牌及製造商如表2，均以一次去離子水稀釋，濃度爲 10%。除了清潔劑外，同時還測試了液態氮、TODD’s DNA buffer 以及 70%、99.5%的酒精溶液等保存媒介。每個測試做3重複，每個重複均包含5 mg (± 0.8 mg)的新鮮組織樣本，樣本分裝在已編號的微量離心管中。編號由 1-1 編至 9-3，前面的數字爲實驗組編號，後面的數字爲該實驗組3個重複樣本中的序號。肌肉組織的分裝過程儘量在冰上操作，避免因處理時間過長而腐敗，造成樣本 DNA 衰退導致實驗誤差。由於已知保存於清潔劑溶液及 TODD’s DNA buffer 的組織樣本在常溫下會自解、均質化(homogenization)於保存液中，因此這兩類保存液均定量於 100 μl，待高溫處理結束後直接將微量離心管全部內容物進行 DNA 萃取程序。酒精保存的樣本則保存於1 ml的酒精中，且在測試開始第2天更換1次酒精溶液，避免保存液濃度被組織樣本本身的水分稀釋。液態氮保存的樣本直接以 2.0 ml 冷凍小瓶(NALGENE®)保存於國立自然科學博物館鳥獸工作室的液態氮保存設備中。選用的組織樣本爲同一隻拉都希氏赤蛙的腿部肌肉組織。所有實驗組的組織樣本均避免沾染血液，以免影響測試結果。切下腿部組織後，青蛙標本以福馬林固定2週，然後轉移至75%的酒精中保存於該館浸液標本蒐藏庫，標本編號爲NMNS#17833。測試的環境條件參考Kuch *et al*. (1999)，測試溫度由 37°C 提高到 50°C，測試時間由14天延長爲28天。除了以液態氮保存的樣本外，其他的樣本以封口蠟膜(Parafilm® M)封住離心管開口細縫後，全數置於電子式恆溫烘箱(Celsius 2000 Memmert)以維持測試溫度，烘箱28天內不間斷連續運作。高溫保存後，樣本進行DNA萃取，操作程序如前述。第1、2、3 組樣本在水浴前先以剪刀將肌肉組織剪碎，以加速樣本均質化程序的進行。

表 2. 各實驗組的相關資訊及測試條件。[1]表示各實驗組的 DNA 濃度平均值相對於第1組樣本(液態氮保存) DNA 濃度的比值

Table 2. Data on preservatives used in the experiment

	Preservatives and brands	Producers	Appearance	Preservation periods and conditions	Crude DNA[1] (%)
1	Liquid nitrogen	翔源行	Transparent liquid	28 days in ultra cold	-
2	99.5% ethanol	島久藥品株式會社	Transparent liquid	28 days in 50°C	81.87
3	70% ethanol (diluted with R. O.)	島久藥品株式會社	Transparent liquid	28 days in 50°C	51.66
4	白蘭濃縮洗衣粉	聯合利華股份有限公司	White grains/ powder mixed with few blue, red grains	28 days in 50°C	77.97
5	加倍潔 SOPP 濃縮洗衣粉	金美克能化學工業股份有限公司	White grains/ powder mixed with few blue, red grains	28 days in 50°C	72.12
6	中性洗潔精 (Neutral cleaner)	Reidel-de Haën®	Sticky transparent liquid	28 days in 50°C	108.19
7	沙拉脫	日星化工股份有限公司	Sticky, light yellow, transparent liquid	28 days in 50°C	83.82
8	泡舒POAS抗菌洗潔精	耐斯企業股份有限公司	Sticky, orange-colored, transparent liquid	28 days in 50°C	25.34
9	TODD's DNA buffer	-	Sticky transparent liquid	28 days in 50°C	128.07

[1] Proportion of DNA concentration equivalent to that preserved in liquid nitrogen.

結果與討論

一、長時間酒精保存的組織樣本DNA衰退情形

由電泳結果(圖 1，表 1)可發現保存在高濃度酒精中 5 年內對樣本的 DNA 影響不大，相對於保存僅 2 個月的樣本，保存 5 年內的樣本萃取出的 DNA 相對濃度約爲 56.99-89.51%；保存 7 年後的樣本crude DNA產量明顯減少，僅 32.52%，且有隨著浸漬時間延長而遞減的趨勢。已保存 9 年的組織樣本可萃取出的DNA質量均差，僅 10.49%。保存 5 年內的樣本DNA濃度並未與時間長短有正相關，可能因各組不同時間點樣本處理時操作上有誤差所致，如所剪取的肌肉組織若較大，樣本瓶中的酒精體積則相對較小，樣本釋出的體液可能造成酒精濃度大幅降低。

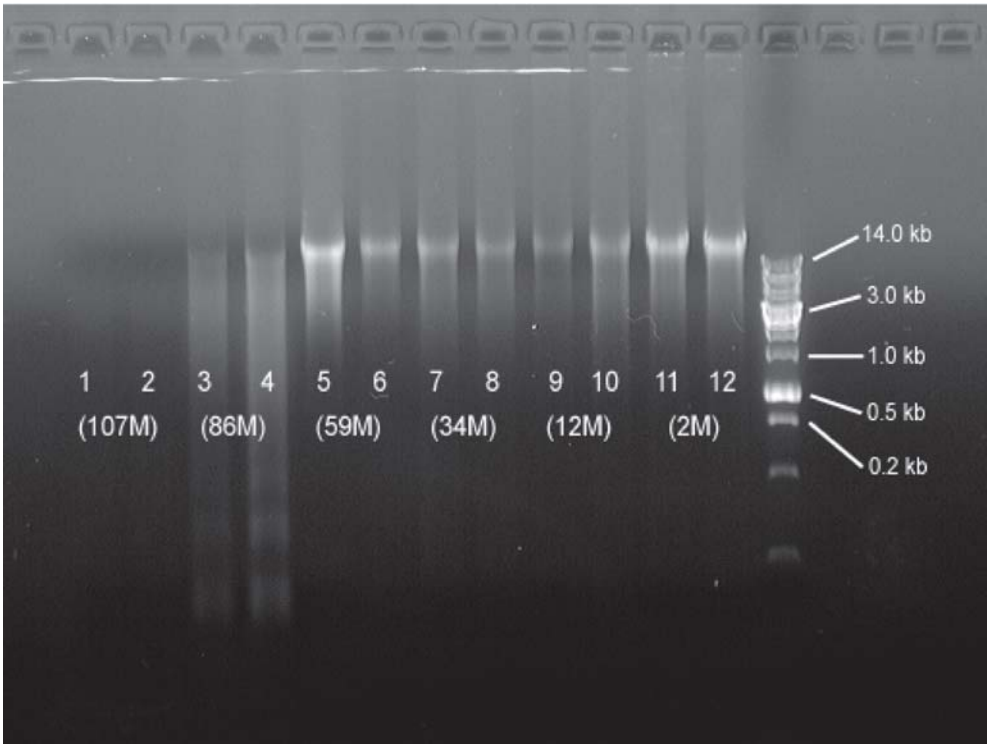

圖 1. 保存於 95%酒精中的 12 組組織樣本 crude DNA 萃取結果電泳圖。電泳凝膠濃度爲 1.2%，每組樣本取 5 μl 測試。樣本保存的時間依次爲 107 個月、86 個月、59 個月、34 個月、12 個月以及 2 個月，每段時間取 2 組樣本。樣本資訊請參考表 1。

Fig. 1. A 1.2% agarose gel showing crude DNA concentrations in 5 μl DNA products from the tissues preserved in 95% ethanol for various periods (M, number of months; data of the specimens 1 to 12 shown in Table 1).

二、利用清潔劑保存的組織樣本 DNA 的效果及限制

10%的兩種廠牌洗衣粉爲過飽和狀態，溶液呈白濁狀；另 3 種廠牌的清潔劑稀釋後皆爲透明液體。除了液態氮及酒精測試組外，其他測試組溶液中的 5 mg 組織樣本在測試溫度下

0.5 hr 內開始有均質化的現象，洗衣粉測試組樣本則有白色沈澱物產生。兩天後第 7、8、9 三組清潔劑溶液中樣本的肌肉組織已經完全均質化。樣本烘烤 28 天後，保存於 99.5%酒精中的樣本(第 2 組)變爲橙紅色，而保存於 70%酒精中的樣本(第 3 組)則呈白色；洗衣粉與清潔劑各實驗組中除中性清潔劑組(第 6 組)外，其餘各組肌肉組織均已均質化於溶液中，中性清潔劑組的肌肉組織則均質化不完全，留有些許半透明狀的組織懸浮其中。兩個洗衣粉溶液實驗組中皆有白色沈澱，應爲溶質過飽和所產生。3 件樣本(3-2、4-3、6-1)在烘烤過程中有部分的肌肉組織沾黏於離心管管壁上未浸漬於測試溶液中，呈乾燥塊狀，未受溶液浸泡。爲避免干擾到測試結果，此 3 組樣本在 DNA 萃取程序進行前即行捨棄。

各實驗組 crude DNA 的產量可由圖 2 推估。除了第 3 組、第 8 組之外，其他實驗組所萃取得到的 crude DNA 質量都相當不錯。由圖 2 得知，TODD’s DNA buffer 組(第 9 組)的產量還多於液態氮保存的樣本(第 1 組)，且較少短片段 DNA 產生。若以第 1 組約 20 kb 大小位置的 DNA 平均產量做爲比較基準，以 AlphaImager IS-2200 影像處理軟體估算各樣本同大小 DNA 片段的平均產量，則還是以 TODD’s DNA buffer 組的 DNA 產量最高，達 128.07%；其次爲第 6 組(中性洗潔精)，達 108.19%。保存於 99.5%酒精中的樣本爲 81.87%。其他實驗組產量表現多在 50%以上，第 3 組(70%酒精)及第 8 組保存效果較差，分別爲 51.66%及 25.34% (表 2)。由此可知，TODD’s DNA buffer 在 50°C 的高溫環境下 1 個月內保存 DNA 的效果其實不遜於液態氮。而多種市面上流通的洗衣粉、洗潔精也可以應用在 DNA 物質的保存，效果直逼昂貴的高濃度酒精。Kuch *et al.* (1999)所測試的 Persil Megaperls®、Persil Supra®及 Liquid Frosch®都是當地相當普遍的家用清潔劑品牌，也成功地證明其在 DNA 保存上的適用性。然而，並非所有的清潔劑都有優異的 DNA 保存效果，本實驗第 8 組(抗菌洗潔精)似乎比 70%酒精的保存效果還差。這提醒我們並非所有的清潔劑都一體適用於 DNA 保存的目的。在利用不熟悉的清潔劑保存 DNA 組織樣本前，最好先進行類似測試，以免誤用不適合的保存溶液而損害珍貴的組織樣本。其他兩種清潔劑，中性清潔劑及沙拉脫，保存效果比第 8 組的抗菌洗潔精好，顯示沒有添加特殊成分的清潔劑比較適用於 DNA 樣本的保存。

三、標本處理及組織樣本保存的建議

與經過福馬林固定處理的標本相比，酒精直接浸泡的動物標本組織可進行 DNA 萃取，進而進行 PCR 增幅、定序等程序，在應用上比福馬林固定過的標本更爲廣泛，適合應用於小型無脊椎動物、魚類或兩棲動物的標本保存。一般浸液標本約保存在 70-80%左右的酒精中(国立科学博物館編 2003; Vink *et al.* 2005)，而要使用於 DNA 萃取、分析的樣本，應以更高濃度的酒精浸漬，以無水實驗級酒精爲佳(Zhang and Hewett 1998)。但是酒精直接浸泡固定的標本容易因脫水而皺縮變形，且酒精濃度越高變形的情形越嚴重，對於外部形態保存的效果不如福馬林。此外，酒精對動物標本的滲透效果也較福馬林爲差，對於體型稍大的動物標本的防腐效果並不理想，特別是內臟的部分，往往在酒精滲透進腹腔之前就已開始腐敗，因而呈現標本肌肉僵硬、腹部柔軟的狀況。直接浸泡在酒精的動物標本體液會逐漸被酒精吸出，稀釋原本的酒精濃度，因此酒精浸漬標本的製作初期要適時更換酒精溶液以維持其濃度。除了有特殊用途(如日後預計取出耳石分析的魚類標本)及披覆外骨骼的無脊椎動物外，對於較大型，或是需要測量其外部形質，或是較爲珍貴稀少的動物浸漬標本，在環境許可的狀況下應以福馬林固定爲優先考量，且在進行固定之前先取下一小部分組織樣本

(如肌肉、肝臟或鰭)另行編號，以低溫冷凍或是浸漬於非福馬林的保存溶液中，如此則可兼顧形態保存及分子生物學方面的應用，將標本發揮最大效益。

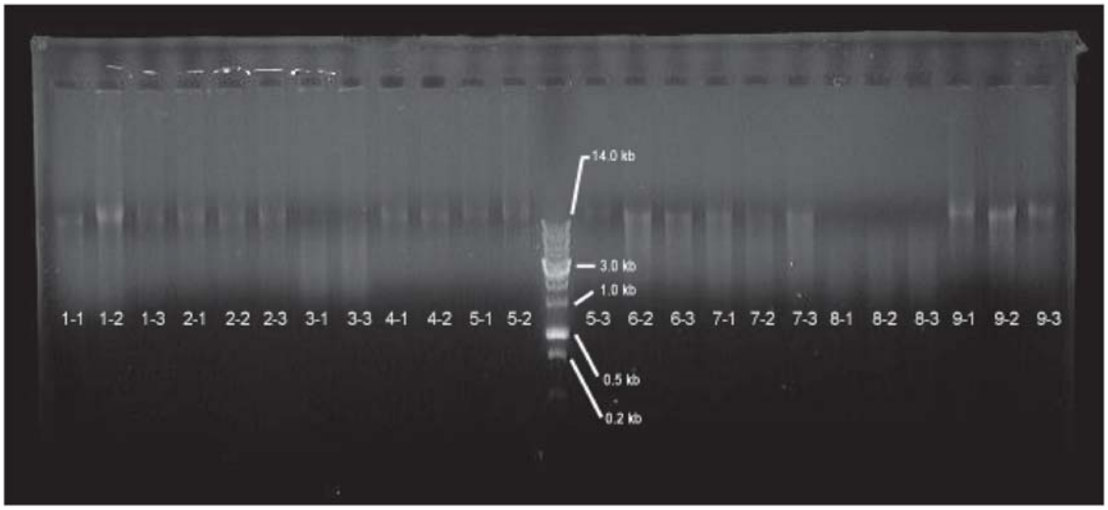

圖 2. 在 9 種介質中保存 28 天後的樣本 crude DNA 萃取結果電泳圖。電泳凝膠濃度爲 1.2%，每組樣本取 5 *μ*l 測試。保存的介質依序爲液態氮(1-1-1-3)、99.5%酒精(2-1-2-3)、70%酒精(3-1 與 3-3)、2 種廠牌的洗衣粉(4-1-5-3)、3 種洗潔精(6-2-8-3)，以及 TODD's DNA buffer (9-1-9-3)。除液態氮組外，其餘各組均保存於 50°C 環境下。各實驗組詳細資料請參考表 2。

Fig. 2. Electrophoreses of crude DNAs in 5 *μ*l DNA products from the tissues preserved with 9 different preservatives for 28 days (1-1-1-3, liquid nitrogen; 2-1-2-3, 99.5% ethanol; 3-1 and 3-3, 70% ethanol; 4-1-8-3, detergents; 9-1-9-3, TODD's DNA buffer; detailed data shown in Table 2).

現今國內各研究單位除了超低溫冷凍設備外，最普遍的組織樣本保存應該還是以酒精浸泡爲主。高濃度酒精以往被認爲是保存樣本DNA物質的首選之一。然而，許多案例(Reiss *et al*. 1995; Hormiga *et al*. 2003; Vink *et al*. 2005)，包括本研究的結果顯示酒精並不是絕無後顧之憂的 DNA 保存媒介。對一般沒有足夠超低溫設備的實驗室而言，面對日漸累積的動物組織樣本，尋得適當的保存方式實爲當務之急。TODD’s DNA buffer 及家用清潔劑似乎是可行的選項。利用清潔劑保存 DNA 組織樣本有著價格低廉、容易取得、非可燃性、操作簡便(一般乾淨的飲用水就可以稀釋)、使用上無法令管制等優點，除了適用於野外工作外，也很適合用來大量保存組織樣本(Kuch *et al*. 1999)。已用酒精固定、保存的組織樣本，爲避免酒精持續破壞樣本的 DNA 結構，應該儘快去除掉酒精，然後直接保存於-20°C 低溫以下的冰箱即可，不用再保存於任何保存液中。若冷凍設備有限，則可利用眞空乾燥機或是低溫烘烤方式將酒精完全去除，然後加入適量(約 20 倍體積)的TODD’s DNA buffer或是清潔劑溶液在室溫下保存。新鮮的組織樣本可在室溫下直接保存於TODD’s DNA buffer中；若在野外長時間採集，特別在無法取得低溫設備、酒精的狀況下，則可購買當地販售的清潔劑充當保存媒介。清潔劑的選用以成分單純、無特殊添加物者爲佳。

TODD’s DNA buffer 或是清潔劑溶液在使用上有個異於酒精的特性，即在室溫或高溫下會溶解樣本組織。動物肌肉組織在室溫下浸泡於 TODD’s DNA buffer，1 天內就可明顯地發現樣本正在分解，剩餘的部分呈半透明狀態。組織溶解後其 DNA 物質會懸浮於保存液中，在室溫中可長期保存。若能在組織完全均質化後移至-20°C 以下的低溫環境保存，則可得更佳的保存效果。懸浮於保存液中的 DNA 物質可很容易地以商用試劑組完成crude DNA的萃取，省略均質化組織的步驟。TODD’s DNA buffer 已被德州大學阿靈頓分校動物學系應用於兩棲爬行動物的組織樣本保存，多年來樣本的保存狀況良好(Eric N. Smith，個人通訊)。

值得注意的是，縱使本研究結果認爲大部分的稀釋清潔劑可以長時間、妥適地保存動物組織樣本的 DNA 結構，然而並非表示可以適用於所有的物種。保存媒介在實際操作時必須考慮到被保存物質的特性，以便做出適當的操作修正。就以常用的酒精來說，雖然普遍應用於各種動物樣本 DNA 的保存中，但是 Zhang and Hewett (1998)曾描述某些甲蟲若一採集到就立刻投入無水酒精中，其 DNA 將被嚴重破壞至無法進行 PCR 增幅程序。若欲以酒精保存那些甲蟲 DNA 樣本，正確的作法是在浸泡於酒精之前先讓甲蟲活著並且餓上幾天，才能成功的保存其 DNA 物質。另外，較大體積的肌肉組織在稀釋清潔液中均質化的過程會比較緩慢，若組織尚未完全溶解前取出保存液進行萃取 DNA 操作，則可能取得的 crude DNA 濃度會顯著低於預期。而像魚鰭等較爲堅固的組織，其硬棘部分不容易在短時間內被清潔劑均質、溶解，保存前最好先用剪刀將其剪碎後再以稀釋清潔劑保存之，當可得到更佳的保存效果。其他如有些動物剛死亡時表皮細胞仍會分泌黏液，有些動物具有毒腺，操作時必須先了解這些物質會不會與保存液相結合，而破壞了 DNA 的結構。在規劃新的研究物種樣本保存計畫時，除了對保存液的適用性要有所了解評估外，也應該先以少量樣本謹慎地測試其可行性，以免危及辛苦得來的整批珍貴樣本。

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附錄、TODD'S DNA buffer 之配製方法

Appendix. TODD'S DNA buffer preparation method

(總體積爲 1.5L)
90g Tris (或是 Tris Base，勿使用 Tris HydroCloride);
+ 3.75g EDTA;
+ 37.5g SDS (Sodium Dodecyl Sulfate);

加純水稀釋至 1.5L，高壓滅菌，分裝至微量離心管或小單位樣本瓶即可。若未及時分裝，在長時間室溫下 SDS 會逐漸析出，分裝前必須先加溫至 SDS 完全溶解。

台灣稀有的唇形科植物—田代氏鼠尾草的再發現

A Rediscovery of *Salvia tashiroi* Hayata (Lamiaceae) from Taiwan

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摘 要

田代氏鼠尾草(*Salvia tashiroi* Hayata)爲唇形科鼠尾草屬植物，日人田代氏 1896 年採於屏東縣恆春，此標本並被早田文藏於 1919 年發表爲新種，此後未有任何採集紀錄；第二版《台灣植物誌》將其列爲未確定種，而在《中國植物志》將其合併爲華鼠尾草之異名。最近於高雄縣六龜鄉十八羅漢山採集到本種，生長於溪谷旁陡坡之開闊地上。本種有單葉和三出複葉兩種形態，此特徵可與台灣本屬其他植物區別。本種與產於中國大陸之華鼠尾草在花部構造和花色上均可區別，應視爲不同的物種。本文描述其分類特徵、生育地和分布，其爲 6 溝花粉，表面具 2 層網狀紋飾，並首次報導本種爲二倍體，染色體數目 $2n = 2x = 16$。

Abstract

In 1896 a single specimen of Lamiaceae was collected by M. Toshiro from Hengchun in the southern Taiwan. A decade later it was described as *Salvia plectranthoides* Griff by Matsumura and Hayata in 1906, but redescribed as a new species *Salvia tashiroi* by Hayata in 1919. For a century, this species has been known only from a single specimen (holotype), and treated as a questionable or uncertaion taxa in the flora of Taiwan, or as a synonym of *Salvia chinensis* Benth. in the flora of China. In our recent plant inventory survey of Taiwan, we rediscovered its wild populations at Mt. Shih-Pa-Lo-Han, Kaohsiung County in the southern Taiwan. This paper confirms the taxonomic status of *Salvia tashiroi* and provides it with a detailed redescription with color photographs, pollen SEM microphotographs, chromosome number (2*n* = 16), and distribution in Taiwan.

關鍵詞：田代氏鼠尾草、唇形科、分類學、台灣

Key words: *Salvia tashiroi*, Lamiaceae, taxonomy, Taiwan

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緒　言

鼠尾草屬(*Salvia* L.)爲唇形科中較爲特化的一群植物，花冠 2 唇，可孕雄蕊 2 枚，藥隔延長成線形，與花絲成丁字著生，約有 900-1,100 種，廣泛分布於舊大陸和新大陸的熱帶和溫帶地區(Li and Hedge 1994)。

西元 1896 年日人田代氏自台灣的屏東縣恆春山中採得 1 份標本(*Y. Tashiro* 20, TI) (圖 1)，此植物有單葉和三出複葉，標本被松村任三和早田文藏於 1906 年鑑定爲長冠鼠尾草(*Salvia plectranthoides* Griff) (Matsumura and Hayata 1906)，但後來早田文藏於 1919 年在《台灣植物圖譜》第八卷中將此標本發表爲新種 *Salvia tashiroi* Hayata，認爲本種特產於台灣(Hayata 1919)。此後未有任何採集紀錄，此學名被後來學者所採用，但均未引證標本(Sasaki 1928; Kudo 1929; Mori 1936)；嗣後本種在台灣迄未見任何其他採集紀錄，可能因爲資料不足，因而在《台灣植物誌》中將其列爲未確定種(Huang and Wu 1978, 1998)；在《中國植物志》將其合併爲華鼠尾草(*Salvia chinensis* Benth.)之異名(Sun and Huang 1977; Li and Hedge 1994)。因此本種植物在台灣地區可以說十分少見，且分類地位仍有待進一步確定。

本文第三作者近來於台灣南部高雄縣六龜鄉十八羅漢山地區採獲此種植物(圖 2)，本文根據文獻、標本和野外實地調查說明本種的分布及其相關特性。

圖 1. 田代氏鼠尾草模式標本(*Y. Tashiro* 20, TI)。

Fig. 1. Type specimen (holotype) of *Salvia tashiroi* Hayata (*Y. Tashiro* 20, TI).

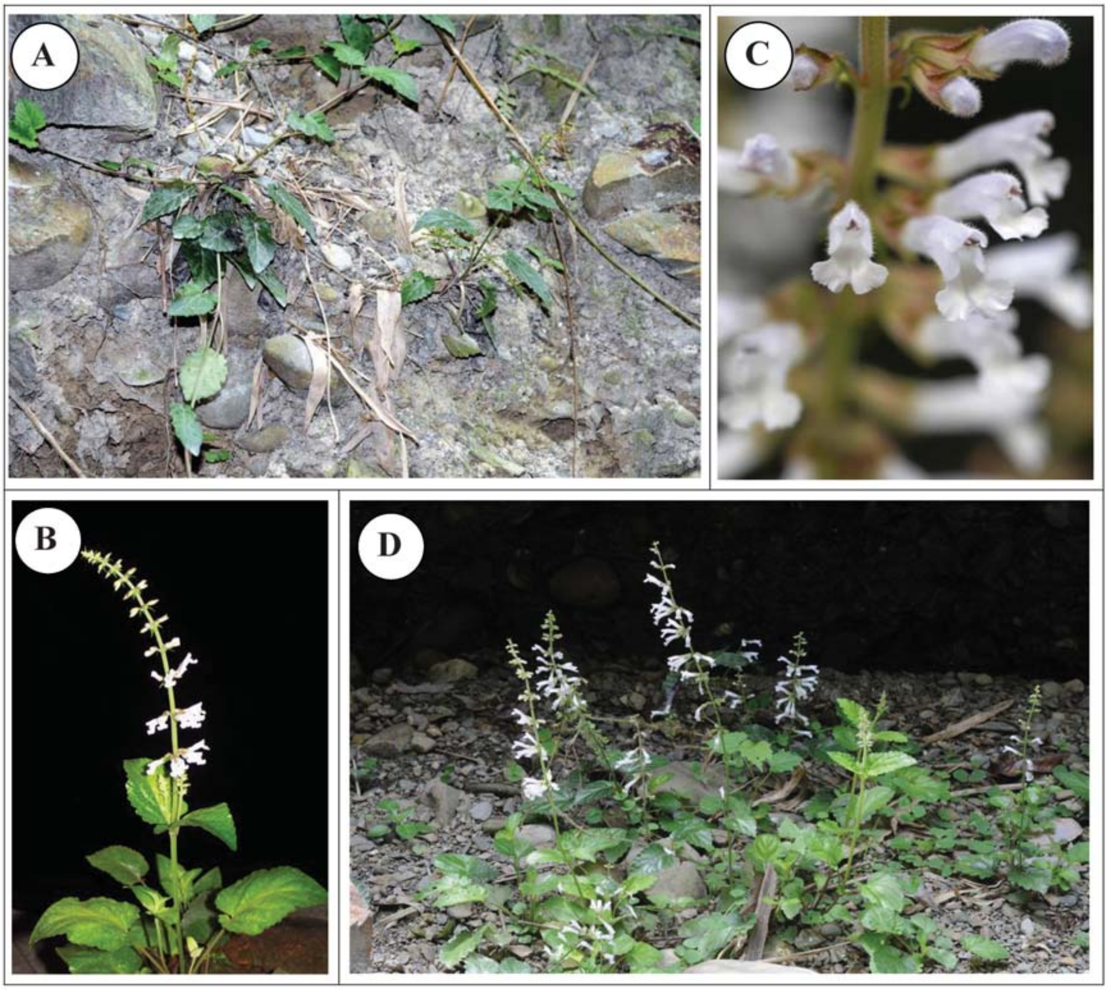

圖 2. 田代氏鼠尾草生育地(A, D)、植株形態(B)和花(C)。

Fig. 2. Habitat (A and D), habit (B) and flowers (C) of *Salvia tashiroi* Hayata.

材料與方法

採自高雄縣六龜鄉十八羅漢山地區的新鮮植物(*T. H. Hsieh et al*. 3005)移植於台南大學蔭棚中栽種，待植株長出新根後，切取根尖約 1 cm 置於 70 ppm cycloheximide 和 250 ppm 8-hydroxyquinone (1:1)混合液中在 18-20°C 下前處理 3-4 hr，接著移至 45% acetic acid 和 absolute ethanol (1:3)混合液中固定 1-3 hr，存放於 70% ethanol 在-20°C 下保存；壓製染色體時將根尖置於 60°C 的 1N HCl 浸泡 1-3 min，在 4% pectinase 溶液中解離 1-2 hr；使用 Sharma 溶液染色 8-10 min (Sharma 1982)，壓片後置於光學顯微鏡(Eclipse E400, Nikon)觀察和照相。

花粉取自新鮮花藥，乾燥後置於離心管中，使用Erdtman的酸分解法(Erdtman 1952)；酸化處理後的花粉粒使用酒精系列脫水，經氣乾後貼於鋁台上鍍金，置於掃描式電子顯微鏡下(3000N, Hitachi)觀察和照相。

結果與討論

在葉形上，田代氏鼠尾草的成熟植株具有單葉和三出複葉(圖 2)，此特徵與台灣本屬其他植物可以區別。台灣本屬植物有些僅具單葉，例如節毛鼠尾草(*S. plebia* R. Br.)和黃花鼠尾草(*S. nipponica* Miq. var. *formosana* (Hayata) Kudo)；有些具有單葉、三出複葉至羽狀複葉，例如阿里山鼠尾草(*S. arisanensis* Hayata)、日本鼠尾草(*S. japonica* Thunb.)和卵葉鼠尾草(*S. scapiformis* Hance)；田代氏鼠尾草幼年期僅具有單葉，成熟植株則會出現三出複葉，但是不會出現羽狀複葉。中國大陸之華鼠尾草亦具有單葉和三出複葉，在葉形上與本種較爲相似。

在習性上，田代氏鼠尾草植株在第 2 年時會從莖基部長出走莖(圖 2A)，在先端節處長出不定根後分株長成新的植株，台灣產本屬植物中僅日本鼠尾草具有類似之特性，但是中國大陸的華鼠尾草爲 1 年生植物，且不具走莖(Sun and Huang 1977)。

在花部構造上，此次在六龜鄉十八羅漢山採集到的植株，長筒狀花冠淡藍或白色，延長伸出花萼之外，長 1.2-1.5 cm，約爲花萼之 3 倍長(圖 2C)；本種原發表時描述的花冠長 11 mm (Hayata 1919)，但是因模式標本中並無開放的花(圖 1)，因而無法重新測量，但從葉部形態確認與本次新採到的植物相同。本種與產於中國的華鼠尾草描述相比較，台灣的標本在花部特徵上有明顯的差異。華鼠尾草花冠爲藍紫或紫色，長約 1 cm，花冠長度與花萼相比約爲 2 倍；台灣的標本花冠爲淡藍或白色，長筒狀，伸出花萼後往上彎曲，長 1.2-1.5 cm，花冠筒明顯的較爲延長。整體而言，台灣的標本與中國大陸的華鼠尾草明顯可以區分。

值得一提的是松村任三和早田文藏於 1906 年曾將本種鑑定爲長冠鼠尾草(*Salvia plectranthoides* Griff)，長冠鼠尾草具有長形的花冠，花冠長約 1.1-2.0 cm，約爲花萼之 3 倍長(Sun and Huang 1977)，兩者的花冠略爲相似；但田代氏鼠尾草具有單葉和三出複葉，花萼內有長粗毛，花冠內面基部有毛環(圖 3)，而長冠鼠尾草具有單葉和羽狀複葉，花萼內無長粗毛，花冠內面基部無毛環，這些特徵都可明確區分此 2 種植物。因此作者認爲台灣的此種植物與大陸有所不同，其最早的合法名爲早田文藏於 1919 所發表的田代氏鼠尾草(*Salvia tashiroi* Hayata)，爲台灣特有種。日本和南洋地區皆未見本種植物之分布(Keng 1969; Murata and Yamazaki 1993)。

本種花粉球形或球狀橫橢圓形，具 6 溝，溝細長，表面具 2 層之網狀紋飾，外層網眼之內有較小之網眼(圖 4)。Huang (1972)曾經研究台灣被子植物之花粉，並報導鼠尾草屬 6 種花粉形態特徵，但並未報導本種。本種花粉的形態和表面紋飾與台灣其他本屬種類頗爲相似。

本文並首次報導田代氏鼠尾草根尖細胞染色體數目 $2n = 16$ (圖 5)，由於本屬的染色體基數爲 8 ($X = 8$) (Huang and Wu 1975)，因此爲二倍體。Huang and Wu (1975)研究本屬植物曾報導有種內二倍體和四倍體的研究，但是本種並未發現四倍體的個體。

性狀描述

田代氏鼠尾草

Salvia tashiroi Hayata, Icon. Pl. Formos. 8: 98. 1919; Sasaki, List Pl. Formos. 360. 1928; Kudo, Mem. Fac. Sci. Agric. Taihoku Imp. Univ. 2: 176. 1929; Mori in Masamune, Short Fl. Formos. 182. 1936. Type: 屏東縣(Pingtung County): 恆春(Hengchun), Apr. 1896, *Y. Tashiro* 20 (Type, TI, photo)。

Salvia plectranthoides auct. non Griff.: Matsum. & Hayata, Journ. Coll. Sci. Univ. Tokyo 22: 311. 1906.

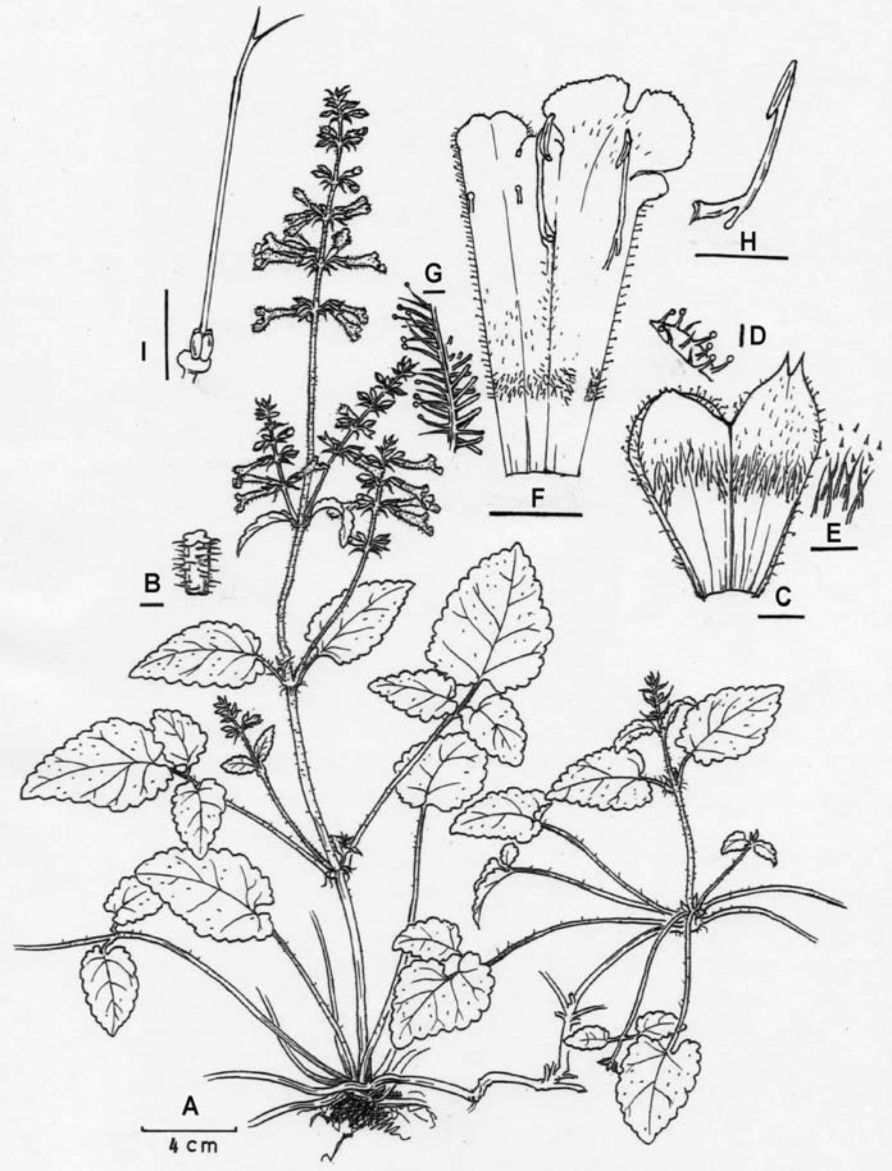

圖 3. 田代氏鼠尾草。A. 習性；B. 莖橫切面；C. 花萼內面；D. 花萼外表之腺毛；E. 花萼裡面之長粗毛；F. 花冠展開圖；G. 花冠表面之腺毛(左側)，裡面具短毛(右側)；H. 雄蕊；I. 雌蕊。比例尺：A = 4 cm; B = 0.3 cm; C = 1 mm; D, E and G = 0.1 mm; F, H and I = 3 mm。

Fig. 3. *Salvia tashiroi* Hayata: A. habit; B. cross section of stem; C. calyx; D. glandular hairs on outer surface of calyx; E. long hairs on inner surface of calyx; F. dissected corolla; G. glandular hairs on outer surface (left) and hairs on inner surface (right) of corolla; H. stamen; I. pistil (scale bars: A = 4 cm; B = 0.3 cm; C = 1 mm; D, E and G = 0.1 mm; F, H and I = 3 mm).

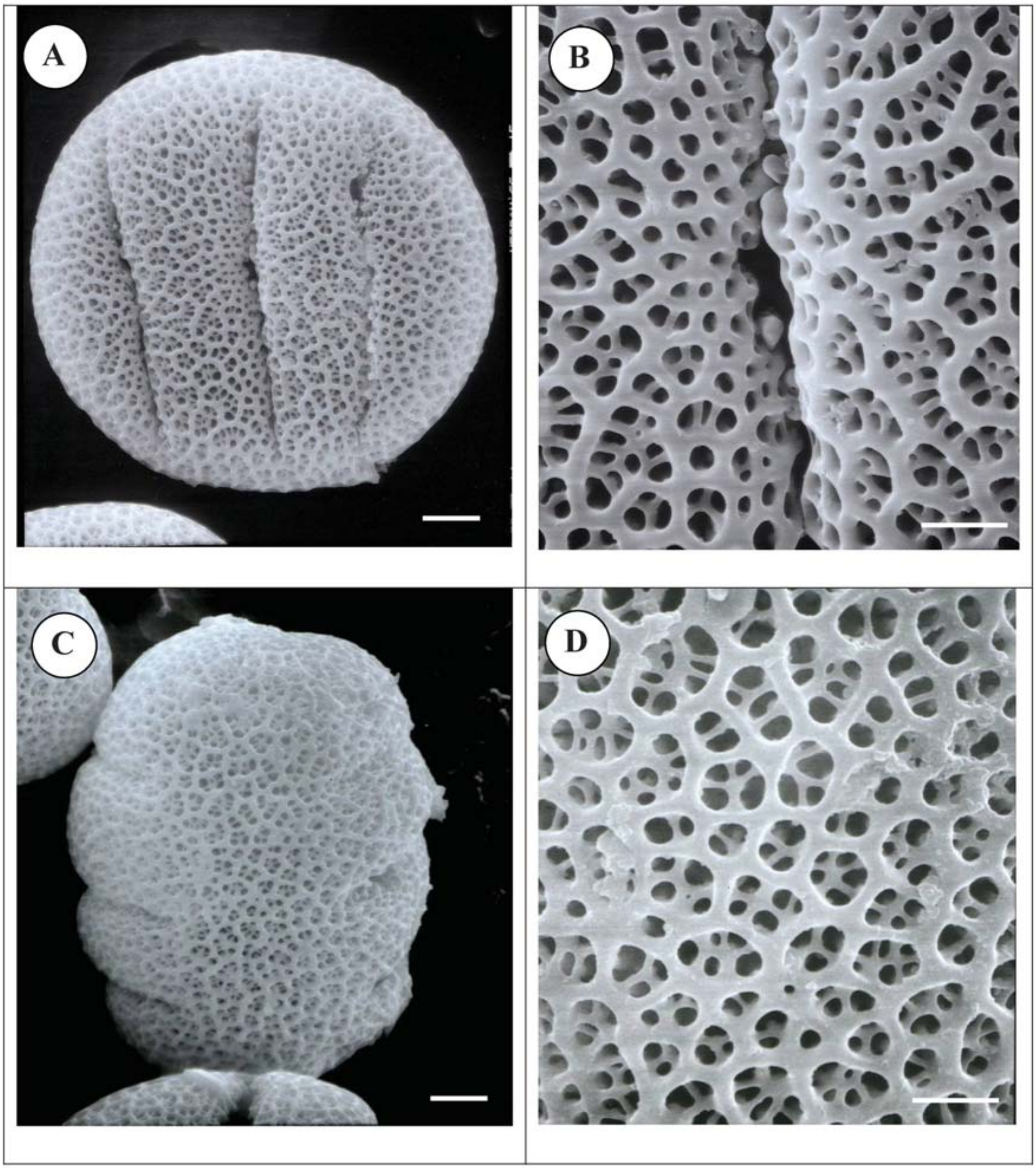

圖 4. 田代氏鼠尾草花粉掃描式電子顯微鏡照相圖。A和B, 花粉赤道面和網狀的外壁紋飾；C和D, 花粉極面 6 溝和網狀的外壁紋飾。A 和 C 之比例尺 = 5 μm，B 和 D 之比例尺 = 1 μm。

Fig. 4. SEM micrographs of pollen grains of *Salvia tashiroi* Hayata: A and B, equatorial views of a pollen grain with reticulate tectum; C and D, polar views of 6-colpate pollen grain with reticulate tectum (bars = 5 μm for A and C; 1 μm for B and D).

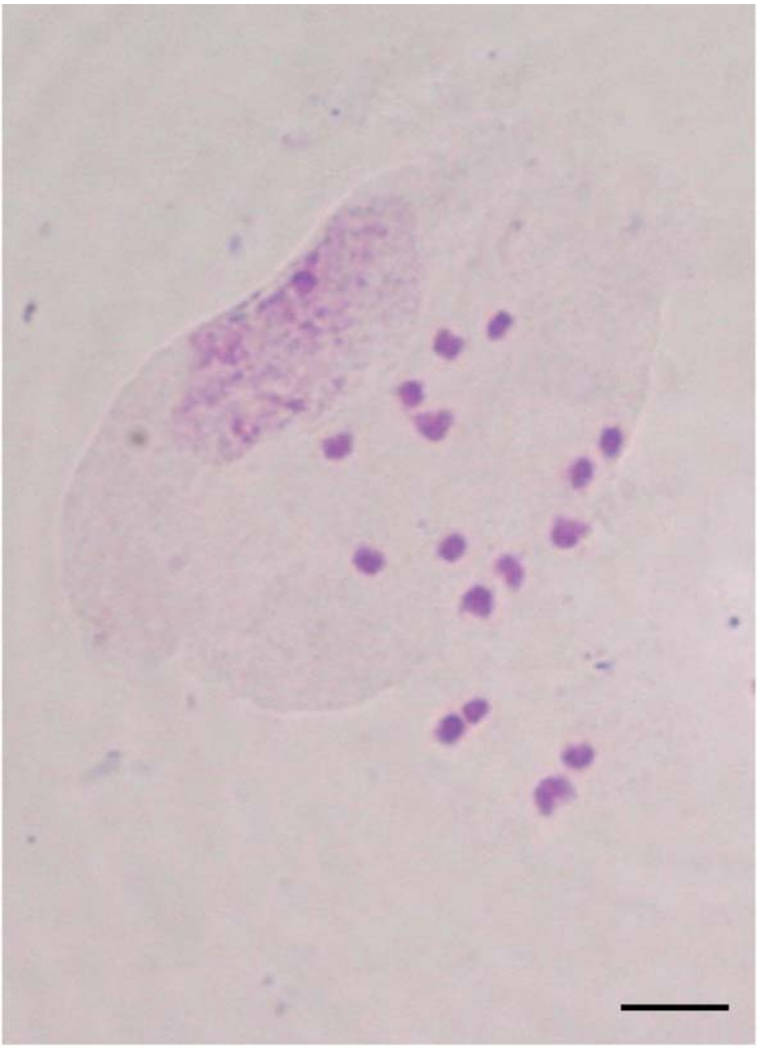

圖 5. 田代氏鼠尾草染色體。根尖細胞染色體數目爲 $2n = 16$。比例尺 = 5 μm。
Fig. 5. Somatic chromosomes ($2n = 16$) of *Salvia tashiroi* Hayata (bar = 5 μm).

1 年生或多年生草本，單一或偶有分枝，高 15-40 cm，基部有時具長走莖，莖四稜，具溝槽，密被短柔毛及稀疏長柔毛，莖與葉柄交接處常具有長柔毛。葉基生和莖生，單葉或三出複葉；基生葉柄較長，6-9 cm，莖生葉柄較短，被短柔毛及稀疏長柔毛。葉片卵圓形或卵圓狀橢圓形，先端鈍或銳尖，基部心形或圓形，葉背有時紫紅色，邊緣圓鋸齒或鈍鋸齒，葉脈被短柔毛，葉面疏被短毛。單葉長 4-7 cm，寬 2.7-4 cm；三出複葉時頂小葉較大，長 3.2-4.2 cm，寬 1.8-2.8 cm，葉柄長 0.6-1.4 cm，側小葉較小，長 1.6-2.7 cm，寬 0.7-1.1 cm，葉柄長 0.1-0.5 cm。每輪具有 6 朵花組成輪生聚繖花序，形成頂生總狀花序，長 10-20 cm；花柄長 1-3 mm，被短柔毛。花萼筒狀，長 4-5 mm，外面沿脈上被短柔毛和腺毛，內部喉部密被長粗毛，萼二唇形，上唇先端 3 淺裂，短尖頭，下唇略長於上唇，長約 0.2 cm，寬約

0.2 cm，半裂成兩齒，齒長三角形，先端漸尖。花冠淡藍或白色，長筒狀，伸出花萼後往上彎曲，長 1.2-1.5 cm，外被短柔毛和腺毛，花冠筒內部離基部 3-4 mm 處披毛環，冠筒長約 1 cm，二唇形，上唇長圓形，先端略凹，下唇三裂，中裂片寬橢圓形，長約 3 mm，先端凹入，邊緣具小圓齒，近中部後收縮，側裂片半圓形；可孕雄蕊 2，著生於冠筒約 7 mm 處，略外伸，花絲短，長約 2-4 mm，花藥 1 室，長約 1 mm，退化雄蕊 2 枚，著生於上唇。花柱底生，長約 1.0-1.3 cm，稍外伸，先端不等長 2 裂。小堅果 4 枚，長橢圓形，橫截面略成三角形，長約 1-2 mm，寬約 8 mm，褐色。

分布：台灣南部低海拔地區，生長於溪谷旁陡坡之開闊地陽光充足處。

觀察標本：高雄縣(Kaohsiung County)：六龜鄉(Liukei)，十八羅漢山(Mt. Shih-Pa-Lo-Han shan)，海拔約 150m，21 May 2009, T. *H. Hsieh et al*. 3005 (NUTN, 台南大學生物科技系標本室)。

註：目前野生族群僅發現於高雄縣六龜鄉十八羅漢山地區，海拔約 150m，此處的山區土壤膠結性較差，侵蝕嚴重，溪谷兩旁因時常崩塌呈陡峭的坡面，大樹生長不易。此區域屬於夏雨型氣候，冬季至翌年春季的乾季長達 4-5 個月(Su 1985)，很多植物不易生長。2008 年 5 月現場調查時發現本種植物可以在乾燥的坡面上生長，有少數植物如槍刀菜(*Hypoestes cumingiana* Benth. & Hook.)、桔梗蘭(*Dianella ensifolia* (L.) DC)、寶島羊耳蒜(*Liparis formosana* Rchb. f.)、南台灣黃芩(*Scutellaria austrotaiwanensis* Hsieh & Huang)等一起生長，但在陡峭坡面極乾處，則只有本種植物生長，現場發現有部分植株葉子呈現下垂凋萎現象，但仍未枯死，且大部分個體處於開花結果之狀態，顯見本種植物相當耐乾旱。推測本種植物可能不耐遮蔭，但較能忍受乾旱，故得以在此種生育地生存。本種在溪谷的砂石堆積處亦可生長，且生長開花良好(圖 2D)，由於此種生育地在雨季時會遭受流水沖刷，植株可能因而流失，雨季過後再從小堅果快速萌發生長。本種耐旱，且容易生長，但可能因爲此種生育地較爲少見，且台灣南部低海拔地區大量被開發，以致目前僅見於此地。

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Distribution of the Freshwater Prawns (*Macrobrachium* Bate, 1868) in Taiwan in Relation to Their Biogeographic Origins

台灣島上淡水沼蝦屬分布與其生物地理起源之關係

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Abstract

Using data collected from the freshwater prawn inventory survey of 2000-2008 and historical data obtained from literature prior to 2000, distribution patterns of 15 species of the *Macrobrachium* prawns in Taiwan were examined. They were able to divide into two groups: the east-coast group and the island-wide group. The east-coast group contained 11 amphidromous species: *M. equidens*, *M. mammillodactylus*, *M. latidactylus*, *M. gracilirostre*, *M. lepidactyloides*, *M. lar*, *M. placidulum*, *M. jaroense*, *M. esculentum*, *M. latimanus* and *M. australe*. They were found in the east coast, some of them in its adjacent northern and southern regions. They were the Southeast Asia origins and dispersed to Taiwan mainly through the Philippines, and showed close association with the Kuroshio Current in the dispersion. The island-wide group contained 4 species: *M. japonicum*, *M. formosense*, *M. asperulum* and *M. nipponense*. The former species was postulated to have evolved within the island of Taiwan, while the latter three species were originated from the China mainland. They were common in streams around the island. *M. japonicum* and

M. formosense that have been considered to be amphidromous showed the distribution patterns fairly similar to that of non-obligatory amphidromous *M. nipponense* and even to that of landlocked *M. asperulum*, rather than to those of the amphidromous species of the east-coast group. The evidences suggest that these two species might not be the amphidromous but the non-obligatory amphidromous, but a further study is needed for the confirmation. The distribution patterns of the *Macrobrachium* prawns in Taiwan are resulted from their life cycle modes, adaptability to local environments at present and biogeographic origins and dispersal routes in the past evolutionary history.

摘 要

台灣 15 種淡水沼蝦在島上的分布範圍，可區分爲東岸型及全島型等 2 種分布類型。東岸型均爲兩側洄游性物種，分別爲 *Macrobrachium equidens*、*M. mammillodactylus*、*M. latidactylus*、*M. gracilirostre*、*M. lepidactyloides*、*M. lar*、*M. placidulum*、*M. jaroense*、*M. esculentum*、*M. latimanus* 及 *M. australe* 等 11 種，牠們分布在台灣的東部，部分物種亦分布在鄰接的北部及南部地區，其中 *M. australe* 則另分布在台灣的西北部。這些物種皆來自東南亞島嶼，並經由菲律賓來到台灣，其在島上的分布及擴散來台的途徑與黑潮關係密切。全島型包括 4 種，分別爲 *M. japonicum*、*M. formosense*、*M. asperulum* 及 *M. nipponense*，前者在台灣種化形成，後 3 種則源自於中國大陸。牠們廣泛分布於台灣的溪流，其中 *M. japonicum* 及 *M. formosense* 爲兩側洄游性，其在島上的分布型態與兼具兩側洄游的 *M. nipponense* 及陸封性的 *M. asperulum* 極爲相似，卻與同爲兩側洄游性的東岸型物種之分布極爲不同，顯示 *M. japonicum* 及 *M. formosense* 或許並非全然爲兩側洄游性，而是如 *M. nipponense* 爲兼具兩側洄游性的物種，此點需要進一步的研究證實。台灣淡水沼蝦在島上的分布型態，是演化過程中物種地理起源、擴散來台途徑及其對環境適應能力等因素所造成。

Key words: biogeography, distribution pattern, *Macrobrachium*, Taiwan

關鍵詞：生物地理、分布型態、沼蝦屬、台灣

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Introduction

Freshwater prawns (*Macrobrachium* Bate, 1868) of Taiwan are highly diverse, rich in amphidromous forms, but poor in endemic and land-locked forms (Hwang and Yu 1982; Shy and

Yu 1998; Cai and Jeng 2001; Cai and Shokita 2006; Chen *et al*. 2009). The fauna is a mixture of the species of various biogeographic origins and more similar to those of the Philippines Islands and the Ryukyu Islands than that of the China mainland (Chen *et al*. 2009).

In the previous study (Chen *et al*. 2009), we examined the distribution patterns of 15 known species of *Macrobrachium* of Taiwan in the Indo-Pacific region and their relationships based on the phylogenies reconstructed from the nuclear 28S rDNA gene. For the 13 amphidromous species, their biogeographic origins and dispersion routes to Taiwan were hypothesized to be: 1) in the eastern region of southeast Asia islands through the Philippines for *M. australe*, *M. lar*, *M. latimanus*, *M. gracilirostre*, *M. jaroense*, *M. esculentum*, *M. lepidactyloides* and *M. placidulum*; 2) in the western region of southeast Asia islands through the Philippines and/or China mainland for *M. equidens*, *M. latidactylus* and *M. mammillodactylus*; 3) in China mainland for *M. formosense*, and 4) on the island of Taiwan for *M. japonicum*. For the remaining two species, *M. asperulum*, a land-locked prawn, and *M. nipponense*, a non-obligatory amphidromous prawn, were originated from China mainland.

This study continued the previous study (Chen *et al*. 2009) to examine the distribution of the *Macrobrachium* prawns on the island of Taiwan and to determine whether there are relationships between the distribution and their biogeographic origins and past dispersion routes.

Material and Methods

During the period from 2000 to 2008, we made an inventory survey of freshwater prawns in inland waters of Taiwan. A 8-volt backpack electrofishing gear (Yeh *et al*. 2000), 30 cm x 10 cm baited shrimp traps (Chen *et al*. 2003), and various types of hand nets (Short 2000) were used. A total of 3,382 individuals belonging to 15 species of *Macrobrachium* were collected at 662 sites. An exception was *M. shaoi* that was extremely rare and found only in a tributary of Shuangchi River in the northern Taiwan (Cai and Jeng 2001) was not collected in this study. Most of the prawns were collected by the shrimp traps, but a lot of large individuals were captured by electrofishing. Most of the collections were fixed in 15% formalin water solution, preserved in 75% ethyl-alcohol water solution, and deposited at the Endemic Species Research Institute, Jiji, Nantou, Taiwan. The data were stored in Microsoft office Access 2003 for database setting. Also, distribution data of the prawns prior to 2000 were obtained from literatures (Hwang and Yu 1982; Shy 1994; Shy *et al*. 1996) and compared to those from our 2000-2008 survey. The distribution map of each species of the prawns was constructed with SuperGIS version 2.2.

Results

Based on field data collected from our 2000-2008 survey and distribution data obtained from literature (Hwang and Yu 1982; Shy 1994; Shy *et al*. 1996), the distribution patterns of 15 species of the *Macrobrachium* prawns in Taiwan were plotted in Figs. 1-4. They were divided into two groups: 1) the east-coast group and 2) the island-wide group:

The east-coast group

The east-coast group contained 11 amphidromous species. They were distributed in the east coast with some in adjacent northern and/or southern regions and absent in the western region (Figs. 1-3). They corresponded to the species of three groups with different biogeographical origins and dispersion routes hypothesized by Chen *et al*. (2009). They were the Eastern Southeast Asia group (*M. esculentum*, *M. gracilirostre*, *M. jaroense*, *M. lepidactyloides* and *M. placidulum*), the Indo-West Pacific group (*M. australe*, *M. lar* and *M. latimanus*), and the Western Southeast Asia group (*M. equidens*, *M. latidactylus* and *M. mammillodactylus*). The former two groups were found in the Philippines but not in China mainland, and thus, they were postulated to originate from the eastern region of Southeast Asia islands and dispersed to Taiwan through the Philippines. The latter group was found in both the Philippines and China mainland and assumed to originate from the western region of Southeast Asia islands and dispersed to Taiwan through the Philippines and/or China mainland (Chen *et al*. 2009).

For the Eastern Southeast Asia group, *M. esculentum*, *M. jaroense* and *M. placidulum* were found to be rare and occurred only in streams along the east coast (Fig. 1), whereas *M. gracilirostre* and *M. lepidactyloides* were common and found not only in the east coast but also in the southwestern region (Fig. 2A-B). The Indo-West Pacific group, *M. lar* and *M. australe* were common and found in the east coast, southwestern and northwestern region of the island (Fig. 2C-D), while *M. latimanus* was rare and occurred in streams of southwestern region and some in the east coast (Fig. 1). For the Western Southeast Asia group, *M. equidens* was common and occurred in the east coast and also in both southwestern and northwestern regions of the island, while *M. latidactylus* and *M. mammillodactylus* were rare and occurred only in the east coast (Fig. 3).

The island-wide group

The island-wide group contained four species: *M. asperulum*, *M. nipponens*, *M. formosense* and *M. japonicum*. They occurred in streams around the island (Fig. 4), differing from those of the east coast group (Fig. 1-3). The former three species are postulated to originate from the China mainland, while the latter species is an autochthonous prawn that was evolved within the island of Taiwan (Chen *et al*. 2009). The four species have been considered to have different life cycle modes: *M. asperulum* as a landlocked prawn (Shokita 1977; Lin *et al*. 1988; Shy 1994; Shy *et al*. 1996; Liu *et al*. 2007; Mashiko and Shy 2008), *M. nipponense* as a non-obligatory amphidromous prawn (Shy *et al*. 1987; Shy 1994; Shy *et al*. 1996; Mashiko and Shy 2008), and *M. formosense* and *M. japonicum* as the amphidromous prawns (Shy *et al*. 1990; Shy 1994; Shy *et al*. 1996; Suzuki and Kusamura 1997; Liu *et al*. 2007; Mashiko and Shy 2008).

Distributions of the freshwater prawns in Taiwan are closed related to their biogeographical origins and modes of their life cycles. The east coast group contained amphidromous prawns of the tropical Southeast Asia islands origins, whereas the island-wide group contained land-locked, non-obligatory amphidromous, and amphidromous prawns of the East Asia origins (Taiwan or China mainland).

Discussion

The east-coast group

Although 11 amphidromous prawns of the east-coast group came from three different

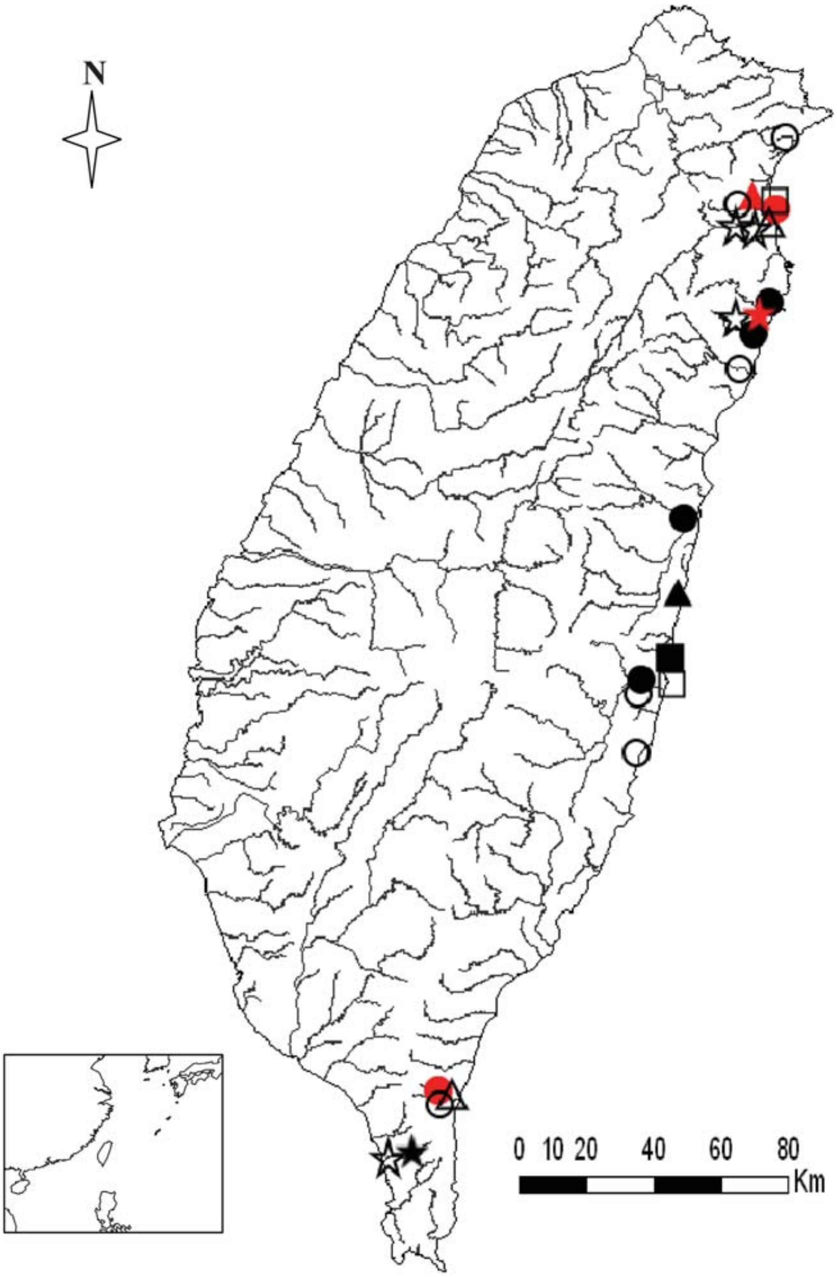

Fig. 1. Distributions of four rare species of the east coast group of *Macrobrachium* in Taiwan: *M. placidulum* (solid circles, 2000-2008 survey; open circles, Shy 1994; red solid circles, Hwang and Yu 1982); *M. jaroense* (solid triangles, 2000-2008 survey; open triangles, Shy 1994; red solid triangles, Hwang and Yu 1982); *M. esculentum* (solid squares, 2000-2008 survey; open squares, Shy 1994); and *M. latimanus* (solid stars, 2000-2008 survey; open stars, Shy 1994; red solid stars, Hwang and Yu 1982).

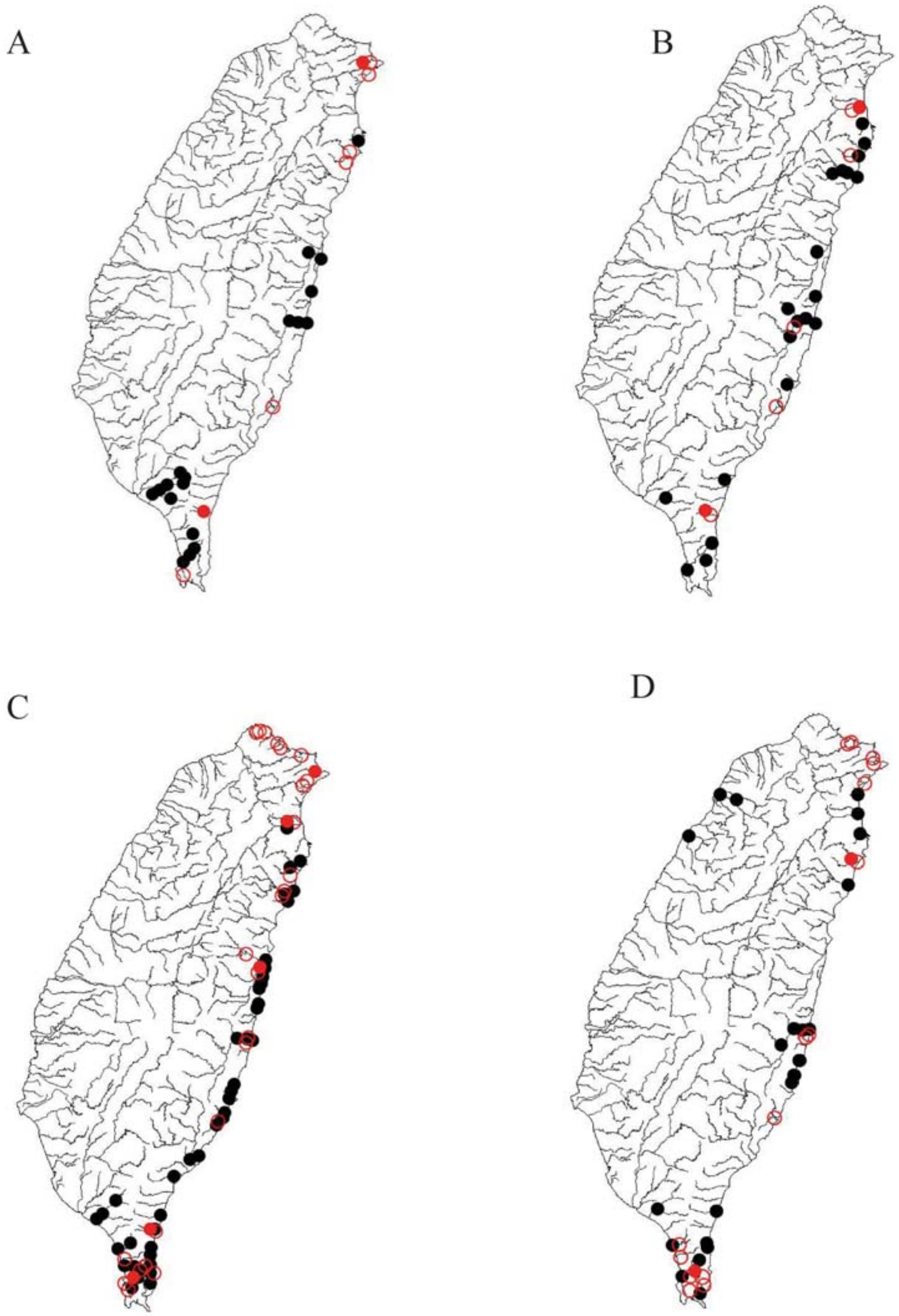

Fig. 2. Distributions of four common species of the east coast group of *Macrobrachium* in Taiwan: A. *M. gracilirostre*; B. *M. lepidactyloides*; C. *M. lar*; D. *M. australe* (solid circles, 2000-2008 survey; red open circles, Shy 1994; red solid circles, Hwang and Yu 1982).

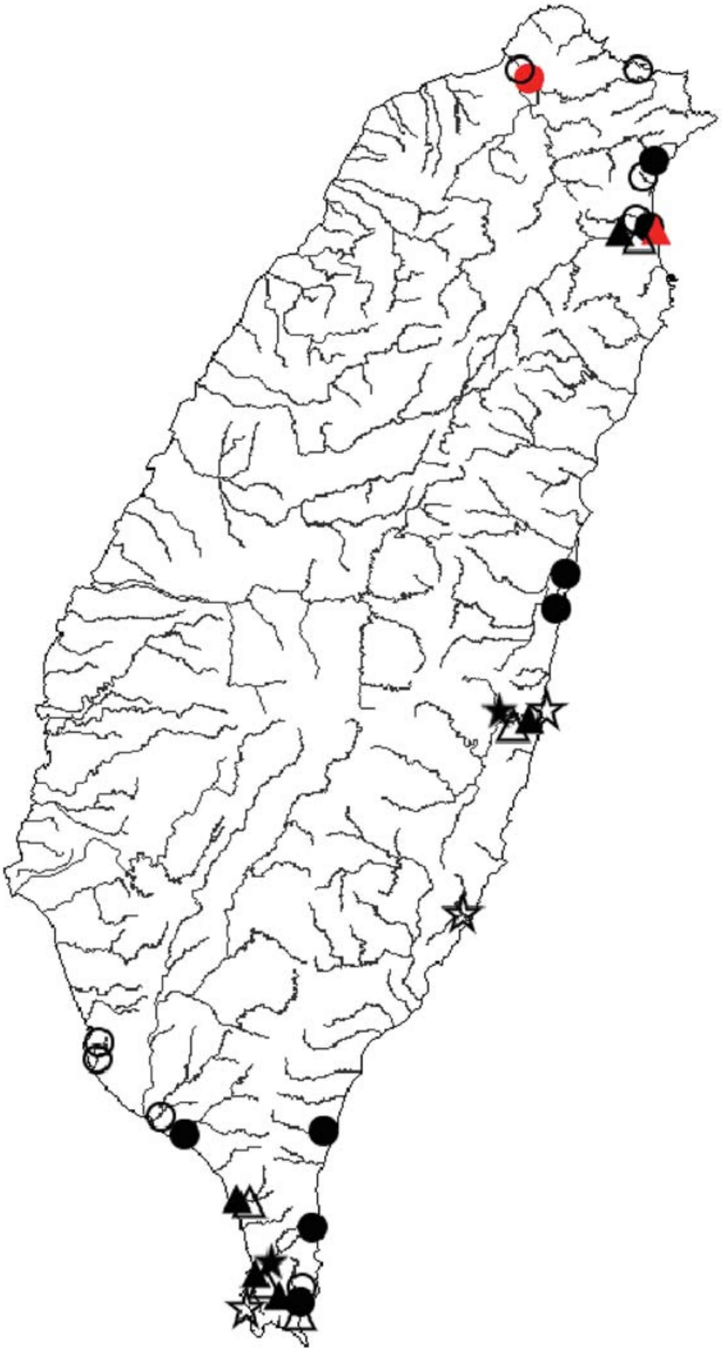

Fig. 3. Distributions of common *M. equidens* (solid circles, 2000-2008 survey; open circles, Shy 1994; red solid circles, Hwang and Yu 1982) and two rare species: *M. latidactylus* (solid triangles, 2000-2008 survey; open triangles, Shy 1994; red solid triangles, Hwang and Yu 1982); *M. mammillodactylus* (solid stars, 2000-2008 survey; open stars, Shy 1994) of the east-coast group in Taiwan.

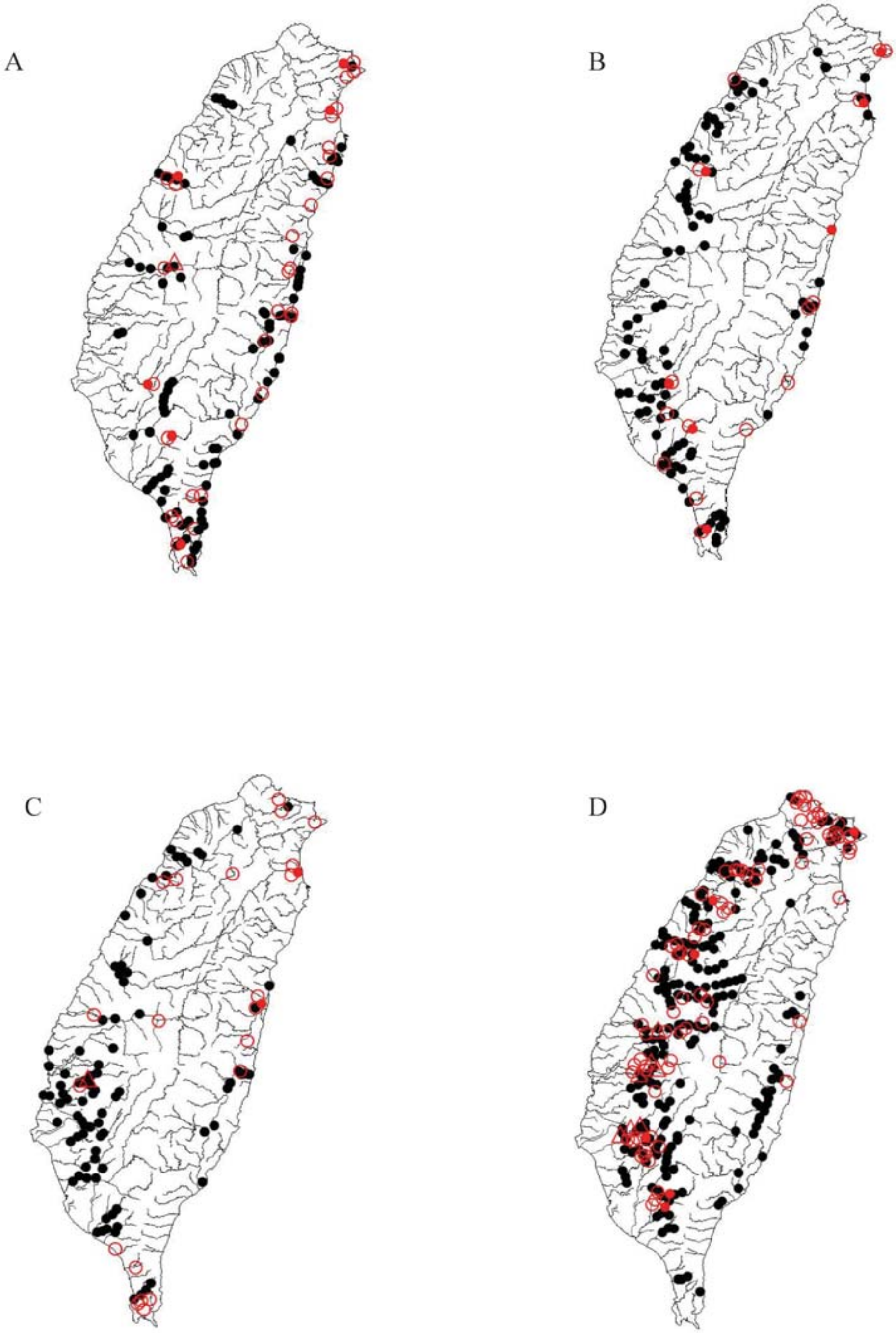

Fig. 4. Distributions of four species of the island-wide group of *Macrobrachium* in Taiwan: A. *M. japonicum*; B. *M. formosense*; C. *M. nipponense*; D. *M. asperulum* (solid circles, 2000-2008 survey; red open circles, Shy 1994; red solid circles, Hwang and Yu 1982; red open triangles, Shy *et al*. 1996).

phylogenetic lineages and biogeographic origins in the tropical Southeast Asia islands (Chen *et al*. 2009), they showed fairly similar distribution pattern in Taiwan. They were distributed in the downstream section of the rivers close to the mouths in the east coast (Figs. 1-3). Apparently, these prawns of Southeast Asia islands origins still shared fairly similar life cycle modes and habitat requirements in Taiwan.

Streams in the east coast of Taiwan have high gradients as the mountain ranges approach closely to the coast. The stream water flows directly into coastal waters, forming a small blackish water zone outside the stream mouth rather than within the mouth. Larval development of these amphidromous prawns must be completed in the coastal waters, suggesting that the larvae may require high salinity and be highly tolerant to it, even to sea water.

There is the warm Kuroshio Current flowing northwardly closely to the shore along the east coast of Taiwan. The larvae of these amphidromous prawns have a chance to be transported northwardly by the current. It seems that the Kuroshio Current might play a role in the northward dispersion of these amphidromous prawns from Southeast Asia islands to Taiwan, even to Japan. According to this line of the reduction, *M. equidens*, *M. latidactylus* and *M. mammillodactylus* of the Western Southeast Asia group that are considered to have dispersed to Taiwan through the Philippines and/or China mainland (Chen *et al*. 2009) might be more likely through the Philippines than through the China mainland.

When the Kuroshio Current hits the southern tip of Taiwan, its main stem flows along the east coast of the island and a small western branch enters the Taiwan Strait and flows along the southwestern coast of the island (Lin *et al*. 1992; Liang *et al*. 2003). This may explain the reason that these tropical prawns are often also found in the southwestern region.

During the Pleistocene glaciation, the western region of Taiwan was connected to the China mainland with the land bridge. The region was undoubtedly inhabitable for amphidromous prawns that requires brackish waster for larval development. Since the last glaciation, the land bridge has been transformed into Taiwan Strait, but the western region has still remained inhabitable for these tropical amphidromous prawns. This may be due to the cold China mainland current that flows southwardly along the west coast from China mainland in winter.

The island-wide group

For the four species of the island-wide group, the distribution patterns of *M. japonicum* and *M. formosense* (Fig. 4A-B) that are considered to be amphidromous (Shy *et al*. 1990; Shy 1994; Shy *et al*. 1996; Suzuki and Kusamura 1997; Liu *et al*. 2007) differed greatly from those of the amphidromous species of the east-coast group (Fig. 1-3) but were fairly similar to that of *M. nipponense* that is non-obligatory amphidromous and *M. asperulum* that is landlocked (Fig. 4C-D).

The life cycles of *M. japonicum* and *M. formosense* are somewhat similar to that of *M. nipponense*. They have small eggs and general nine zoea stages (Ogasawara *et al*. 1979). In Japan the three species are found to spend their long pelagic zoeal stage in estuarine brackish waters, and then juveniles of *M. formosense* and *M. japonicum* migrate upstream to freshwater (Ogasawara *et al*. 1979; Shokita 1979; Shy *et al*. 1990), whereas individuals of *M. nipponense* remain in the estuaries (river mouths) to complete their life cycle, but some populations are found in coastal or inland freshwater lakes, resulting from a recent shift in its habitat from estuaries to inland freshwaters (Mashiko 1990; Mashiko and Numachi 2000). Such shift to freshwater form from amphidromous form have been well documented for other freshwater shrimp *Paratya australiensis* Kemp, 1917 in Australia (Williams 1977; Hancock and Bunn 1977; Walsh and Mitchell 1995; Cook *et al*. 2006), and also many species of fishes and invertebrates (Lee and Bell 1999; Lee 1999; Taylor and McPhail 1999; Waters and Wallis 2001; Raeymaekers *et al*. 2005; Cook *et al*. 2006). The shift of amphydromy to freshwater form plays an important role in diversification of many freshwater fauna in evolution.

The above evidences may suggest that *M. japonicum* and *M. formosense* might not be amphidromous but non-obligatory amphidromous like *M. nipponense*. A further study is needed to confirm the life cycle modes of those two species.

Mashiko and Shy (2008) used molecular clock to estimate the time of speciation events and suggest that *M. shokitai* endemic to the Irimoto Island of the southern Ryukyu differentiated from *M. asperulum* of Taiwan approximately 1.0 million years ago, and *M. nipponense* from *M. formosense* 0.48 million years ago. According to Lee (2006) the uplift rate of Taiwan Central Range was initially slow at a rate of < 1 mm/year from 6 million years ago to 1 million years ago. Since then the rate has increased to 4-10 mm/year. The mountains started to build from the north toward the south at a rate of 60-90 km/million years.

M. asperulum of China mainland might arrive in the west coast of Taiwan before the time of the rapid uplift of the Central Mountain Range 1 million years ago at the time when the east coast remained in connection to the west coast with shallow valleys. This might enable the species to disperse across the island to the east coast. *M. nipponense* and *M. formosense* arrived in the west coast of Taiwan from China mainland apparently latter than that of *M. asperulum*, but still enable them to disperse to the east coast. The similar cases of cross island dispersions are reported for the freshwater fish *Varicorhinus barbatulus* and freshwater crab *Candidiopotamon rathbunae* (Wang *et al*. 2004; Shin *et al*. 2006).

The distribution patterns of the *Macrobrachium* prawns in Taiwan are related to their biogeographic origins and dispersal routes in the evolutionary history, their life cycle modes, and their adaptability to local climatic and environmental conditions at the present.

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New Records of a Branchiobdellidan and Four Microdrile Oligochaetes (Annelida: Clitellata) from Inland Waters of Taiwan

台灣內水域新紀錄一種蛭蚓類及四種貧毛類

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Abstract

In addition to known host *Neocaridina* spp., this paper reports the first record of the ectosymbiont branchiobdellidan *Holtodrilus truncatus* (Clitellata: Branchiobdellida) on *Caridina pseudodenticulata* (Decapoda: Atyidae) from Taiwan. Both host shrimp species occurred sympatrically at collection sites, suggesting that *H. truncatus* has no species specific preference for host. Eight species of free-living microdrile oligochaetes were also collected from the collection sites. Of these, four species, *Nais pardalis*, *Pristina proboscidea*, *Pristina amphibiotica* and *Aulodrilus japonicus*, are new records to Taiwan.

摘　要

本研究描述1種台灣新紀錄的蛭蚓類*Holtodrilus truncatus* (Clitellata, Branchiobdellida)，其宿主除已知的 *Neocaridina* spp. 外，首次發現亦寄宿在與 *Neocaridina* spp.共域棲息的 *Caridina pseudodenticulata* (Decapoda, Atyidae)，因此 *H. truncatus* 並無宿主專一性。另外亦採集到8種自

由營生性的貧毛類，其中4種*Nais pardalis*、*Pristina proboscidea*、*Pristina amphibiotica*及*Aulodrilus japonicus* 爲台灣新紀錄種。

Key words: Clitellata, Branchiobdellida, oligochaetes, Taiwan, freshwater

關鍵詞：環節動物門、蛭蚓目、貧毛類、台灣、淡水

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Introduction

Branchiobdellidans are obligatory ectosymbionts, living mostly on freshwater astacoidean crayfishes in the Holarctic region, but some are on other crustaceans as their hosts (Gelder 1999; Brinkhurst and Gelder 2001). Such branchiobdellidans on non-crayfish hosts in East Asian include *Caridinophilus unidens* Liang, 1963 living on the freshwater atyid *Caridina* shrimp (Liang 1963) in Yunnan Province, and of *Holtodrilus truncatus* (Liang, 1963) on *Neocaridina* shrimp in Hunan and Guangdong Provinces (Liang 1963; Liu 1984) in China. A similar symbiotic association between branchiobdellidans and freshwater shrimps has been reported from the central Japan by Niwa *et al*. (2005) and Niwa and Ohtaka (2006). Recently, a branchiobdellidan tentatively identified as *H. truncatus* has been found to live on three indigenous *Caridina* shrimp species on Miyako Island of Ryukyu in the southern Japan (Fujita *et al*., unpubl.). Therefore, it has been postulated that branchiobdellidans might also occur in nearby Taiwan, as the island is rich and abundant in freshwater atyid shrimps and close geographical proximity to both southern China and southern Japan.

Studies on taxonomy and diversity of oligochaetes in Taiwan and its adjacent islands have been focused on terrestrial forms, and 72 species and subspecies of megadriles (earthworms) have been reported (Tsai *et al*. 2009). On the other hand, 26 species of aquatic microdrile oligochaetes comprising 25 naidids and one enchytraeid have been recorded from Taiwan (Cheng 1995; Yu *et al*. 1995; Erséus and Hsieh 1997; Hsieh *et al*. 1998, 1999; Lin *et al*. 2005; Lin and Yo 2008a, 2008b; Lin *et al*. 2008). These aquatic forms have been found in lowland areas including brackish estuaries and urban rivers mainly from pollution and ecologically related studies.

When we collected freshwater shrimps in searching for branchiobdellidans in Taiwan, we also collect benthic samples to identify free-living freshwater microdrile oligochaetes. This paper briefly describes our findings on branchiobdellidans and microldrile oligochaetes collected from Taiwan.

Material and Methods

Branchiobdellidan and microdrile oligochaete specimens were collected from two streams and one pond in the northwestern part of Taiwan (Fig. 1). The branchiobdellidans were collected from shrimps sampled, and the oligochaetes were collected with a dipnet (mesh size, 190 μm) by scooping it through aquatic vegetation and bottom sediments. Substrate samples and shrimp specimens were fixed in a 10% formalin solution at collection sites. In laboratory the oligochaetes were separated from the sediments and the branchiobdellidans from the shrimps under a dissecting microscope. The specimens were washed thoroughly in distilled water, dehydrated in a graded series of ethanol-water solutions, cleared in methyl salicylate, and mounted in Canada balsam on slides. Some of the slide-mounted specimens are deposited at the Endemic Species Research Institute (ESRI).

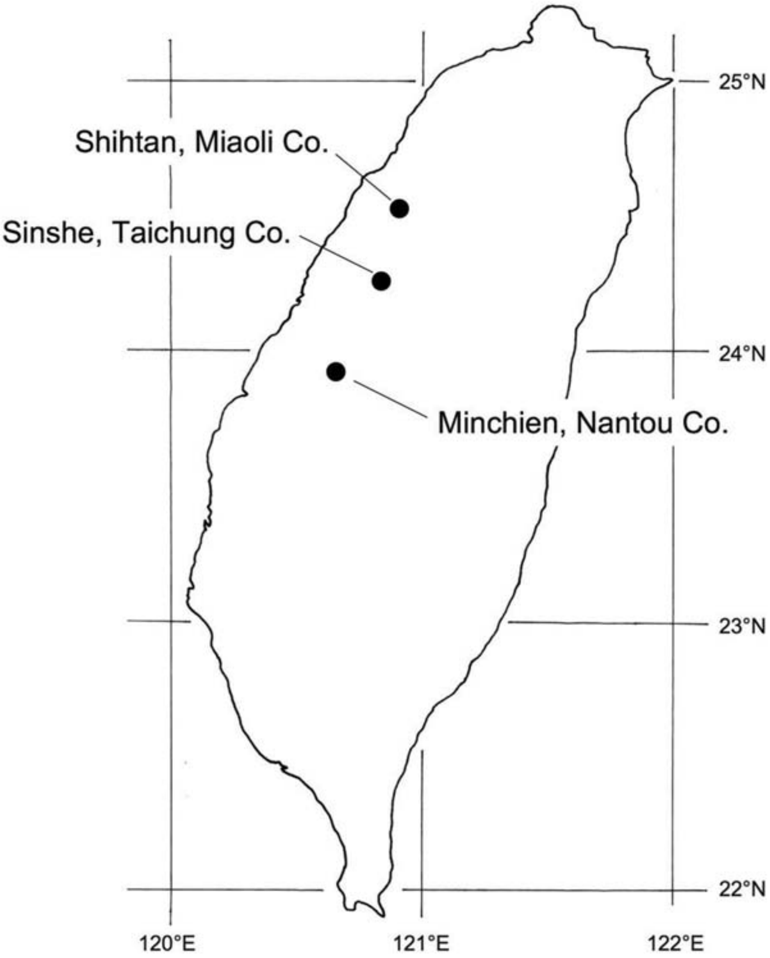

Fig. 1. A map of Taiwan showing the collecting sites (solid circles).

The Taiwanese *Neocaridina* shrimp has traditionally been identified as *N. denticulata denticulata* (Kubo 1938), but its' taxonomic (specific or subspecific) status remains controversial (Cai 1996; Liang 2004; Nishino and Niwa 2004; Shih and Cai 2007). Therefore, we regarded the shrimps as *Neocaridina* spp. Taxonomy of families and subfamilies of the oligochaetes follows the classification system of Erséus and Gustavsson (2002) and Erséus *et al*. (2008). In descriptions of species, segmental numbers are expressed by Arabic numerals for branchiobdellidans but by Roman numerals (= ganglionic numbers) for aquatic oligochaetes, according to the currently accepted usages for the two taxa (Brinkhurst and Gelder 2001).

Systematic accounts

Class Clitellata

Order Branchiobdellida

Family Branchiobdellidae Grube, 1851

Holtodrilus truncatus (Liang, 1963) (Fig. 2)

Stephanodrilus truncatus Liang, 1963: 565-567, fig. 4.

Holtodrilus truncatus Gelder and Brinkhurst, 1990: 1320-1321, fig. 3; Niwa *et al*., 2005: 685-686, fig. 1; Niwa and Ohtaka, 2006: 182-185, fig. 2.

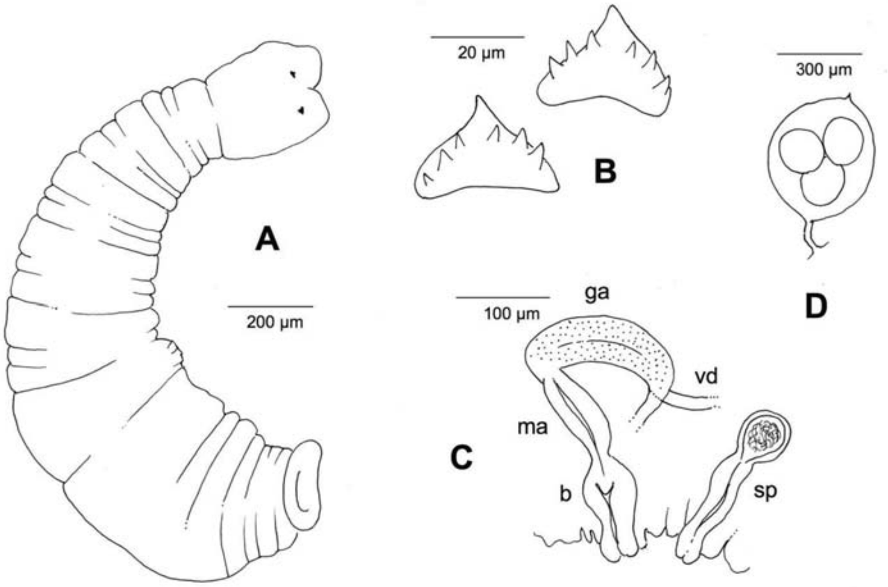

Fig. 2. *Holtodrilus truncatus* from Sinshe, Taichung Co., Taiwan: A. lateral view of whole specimen (fixed); B. jaws; C. genital organs; D. cocoon; b, muscular bursa; ga, glandular atrium; ma, muscular atrium; sp, spermatheca; vd, vas deferens.

Material examined. - Twenty mature specimens (one of the slide mounted specimens was deposited at ESRI as ESRI-OA0001) from 90 host shrimp *Neocaridina* spp. collected on 10 September 2008 from a stream with a gravel-sandy bottom and submerged vegetation dominated by *Egeria densa* Planch in Sinshe, Taichung County (24°14'N, 120°48'E); 10 mature and 20 immature specimens from 12 *Neocaridina* spp. and 61 *Caridina pseudodenticulata* Hung *et al*., 1993, collected on 11 September 2008 from a stream with a gravelly bottom but no vegetation, Miaoli County (24°28'N, 120°53'E).

Brief description. - Living primarily in branchial chamber of host. Body terete, brownish transparent when living. Body length (mature) 1.3-2.6 mm in fixed state (Fig. 2A). No peristomial lobe or dorsal segmental appendage. Dorsal lip slightly longer than the ventral. Head 0.20-0.28 mm in width, wider than that of segment 1. Posterior attachment disc 0.16-0.21 mm in diameter. Segments 1-6, triannulate, middle annulus shortest. Dorsal and ventral jaws similar in shape and size; each triangular, about 25-35 µm in width at base, with a large median tooth and three pairs of small lateral teeth, sometimes one lateral tooth missing or added (Fig. 2B). Two vasa deferentia entering glandular atrium entally in segment 6 (Fig. 2C). Glandular atrium tubular, 180 µm in length and 50 µm in diameter, prostate gland absent. Muscular atrium terete, 110 µm in length, leading to conical penis surrounded by muscular bursa. Spermatheca in segment 5, duct 130 µm long with a spherical bulb, 50 µm in diameter (Fig. 2C). Cocoons spherical to ovoid in shape, 0.4-0.6 mm in height and 0.35-0.55 mm in width, with a small process dorsally and peduncle cemented onto gill surface (Fig. 2D).

Remarks. - *H. truncatus* was originally placed under the genus *Stephanodrilus* Pierantoni, 1906 (junior synonym of *Cirrodrilus* Pierantoni, 1905) on the host shrimp *Neocaridina denticulata sinensis* (Kemp, 1918) from Sichwan in Hunan Province of China (Liang 1963). It was subsequently reported from Shaoguan in Guangdong Province from the same species of the host shrimp by Liu (1984). Gelder and Brinkhurst (1990) transferred the species to a new monotypic genus *Holtodrilus*, because of its unique taxonomic characters. Morphological characters of the specimens collected from Taiwan agreed closely with those described by Liang (1963) and Gelder and Brinkhurst (1990). This study confirmed that *H. truncatus* lives primarily in branchial chamber of the host shrimp as noted by Liu (1984) in China.

This paper reports the first record of *H. truncatus* from Taiwan. Besides it was found on *Neocaridina* shrimps (Liang 1963; Liu 1984), it was also found on a new host *Caridina pseudodenticulata*. *H. truncatus* was found at two out of the five sampling sites where *Neocaridina* and/or *Caridina* shrimps were found together. These two shrimps occurred sympatrically at Shihtan, Miaoli County where *H. truncatus* and its cocoons were found on both shrimps. This phenomenon was anticipated as branchiobdellidans are known to live on many species of crayfishes (Gelder *et al*. 2001).

When the North American red swamp crayfish *P. clarkii* (Girard, 1852) carried the branchiobdellidan *Cambarincola mesochoreus* Hoffman, 1963 was introduced into northern Italy, *C. mesochoreus* was later found on the sympatric native crayfish *Austropotamobius pallipes* (Lereboullet, 1858). In turn, two endemic branchiobdellidans, *Branchiobdella hexodonta* Gruber, 1883 and *Branchiobdella parasita* Henle, 1853, on native *A. pallipes* were also found later on the introduced *P. clarkii* (Gelder *et al*. 1999).

In Yunnan Province of China, a shrimp-associated branchiobdellidan *Caridinophilus unidens* was recorded from *Caridina yunnanensis* Yu, 1938 by Liang (1963). Recently, a branchiobdellidans tentatively identified as *H. truncatus* on Miyako Island, Japan, was found on three sympatric *Caridina* shrimps: *Caridina rubella* Fujino and Shokita, 1975, *C. rapaensis* Edmondson, 1935, and *C. typus* H. Milne Edwards, 1937 (Fujita *et al*., unpubl.). Brinkhurst and Gelder (2001) suggest that all crayfishes appear to be potential hosts for branchiobdellidans. A similar case of the shrimp-branchiobdellidan association was found in Taiwan. It still remains a question whether the Taiwanese population of *H. truncatus* is native or introduced.

Order Tubificida

Family Naididae Ehrenberg, 1828

Subfamily Naidinae Ehrenberg, 1828

Chaetogaster diaphanus (Gruithuisen, 1828)

Nais diaphana Gruithuisen, 1828; see Brinkhurst and Jamieson (1971) for synonyms and references.

Material examined. - One immature specimen (ESRI-OA0002) and another immature specimen collected from a stream in Sinshe, Taichung County (same collection data as for *H. truncatus*).

Brief description. - Body transparent, 0.8-1.5 mm long. Number of segments in zooids 9. Prostomium inconspicuous with narrow and truncated anterior end in fixed state. Dorsal chaetae absent. Ventral chaetae 6-8 per bundle in segment II, 144-190 µm in length, longer than those in VI; each with nodulus proximally, with upper tooth longer but as thick as the lower tooth; absent in III-V; 4-6 per bundle in VI, 62-74 µm long, with nodulus medially and longer upper tooth.

Remarks. - *C. diaphanus* is characterized by having long ventral chaetae in segment II. It is a common and widely distributed species found not only in freshwater but also in brackish water environments (Brinkhurst and Jamieson 1971). In Taiwan, this species was recorded from Taichung City by Lin and Yo (2008a).

Nais pardalis Piguet, 1906

Nais bretscheri var. *pardalis* Piguet, 1906; see Brinkhurst and Jamieson (1971) for synonyms and references.

Material examined. - One immature specimen (ESRI-OA0003) and another immature specimen were collected from a stream in Sinshe, Taichung County (same collection data as given for *H. truncatus*).

Brief description. - Body length 1.2-2.0 mm in fixed state. Number of segments in zooids 16. Prostomium rounded. Eyes present. Dorsal chaetae starting from segment VI and consisting of a 96-144 µm long smooth hair and a 45-56 µm long needle. Needles in bayonet-shape with nodulus distally and two fine, parallel, teeth of equal sized. Ventral chaetae 2-4 per bundle in II-V, 70-83 µm in length, longer, thinner and straighter than those in the following segments, with median or proximal nodulus, and upper tooth longer and thinner than lower tooth; in the following segments 2-5 per bundle, 61-72 µm long, with distal nodulus, and upper tooth as long as or slightly shorter but thinner than lower tooth.

Remarks. - Although *N. pardalis* has ventral chaetae in segments after VI, that are often enlarged with longer upper teeth (Brinkhurst and Jamieson

1971), the two specimens from Taiwan have normal chaetae only. It is a cosmopolitan naidine species. (Brinkhurst and Jamieson 1971). *Nais pardalis* reported herein constitutes the first record of this species from Taiwan.

Dero digitata (Müller, 1773)

Nais digitata (*coeca*) Müller, 1773; see Brinkhurst and Jamieson (1971) for synonyms and references.

Material examined. - Three immature specimens (one slide specimen deposited as ESRI-OA0004) collected from mud surface from a vegetable farm pond in Minchien, Nantou County (23°52'N, 120°42'E); one immature specimen from a stream in Sinshe, Taichung County (same collection data as for *H. truncatus*).

Brief description. - Body length 4-10 mm in fixed state. Number of segments in zooids 24. Prostomium bluntly conical. No eyes. Dorsal chaetal bundles beginning in segment VI, consisted of a 160-240 µm long smooth hair and a 64-77 µm long bayonet-shaped needle. Needle chaetae with distal nodulus and short bifurcate teeth; upper tooth longer than but as thick as lower tooth. Ventral chaetae 4-6 per bundle in II-V, 104-118 µm in length, longer and thinner than those in the following segments; each with proximal nodulus and long bifurcated distal teeth of which upper tooth longer, than and as thick as lower tooth. Ventral chaetae in the following segments, 3-5 per bundle, 71-88 µm long, with distal nodulus and upper tooth as long as and thinner than lower tooth. Branchial fossa has 4 pairs of gills.

Remarks. - *D. digitata* is a cosmopolitan species (Brinkhurst and Jamieson 1971). It was recorded from Taichung County, Taiwan by Lin and Yo (2008a).

Pristina proboscidea Beddard, 1896

Pristina proboscidea Beddard, 1896; see Brinkhurst and Jamieson (1971) for synonyms and references.

Material examined. - One immature specimen (ESRI-OA0005) and two immature specimens collected from a stream in Sinshe, Taichung County (same collection data as for *H. truncatus*).

Brief description. - Body length 1.5-2.0 mm in fixed state. Number of segments in zooids 14-16. Prostomium forming a proboscis. No eyes. Dorsal chaetal bundles beginning in segment II, consisting of hairs and needles. Doral hairs 2-4 per bundle, 192-230 µm long, serrated. Dorsal needles straight, 2-3 per bundle, 40-48 µm long, simple-pointed, without nodulus. Ventral chaetae 5-8 per bundle, 56-64 µm long, with nodulus medially to distally; upper tooth longer and as thick as or slightly thinner than lower tooth.

Remarks. - *P. proboscidea* has been recorded from South America, Africa, South and East Asia, and Australia (Brinkhurst and Jamieson 1971). In Southeast Asia its predominant habitat was recorded as submerged vegetation from Central Kalimantan, Indonesia (Ohtaka *et al*. 2006). This paper gives the first record of this species from Taiwan.

Pristina amphibiotica Lastockin, 1927

Pristina amphibiotica Lastockin, 1927; see Brinkhurst and Jamieson (1971) for synonyms and references.

Material examined. - One immature specimen

(ESRI-OA0006) collected from stream bed in Sinshe, Taichung County (same collection data as for *H. truncatus*).

Brief description. - Body length 1.3 mm in fixed state. Number of segments in zooids 23. Prostomium conical with rounded apex without proboscis. No eyes. Dorsal chaetal bundles starting in segment II, consisted of hair and needle chaetae. Dorsal hairs smooth, 1 or occasionally 2 per bundle, 105-256 µm long. Dorsal needles in bayonet-shape, 1-2 per bundle, 48-64 µm long, with distal nodulus and parallel teeth of which upper tooth distinctly shorter and much thinner than lower ones. Ventral chaetae 4-6 per bundle, 46-56 µm long; those in anterior segments with a little distal nodulus and upper tooth a little longer but as thick as lower tooth; those in posterior segments with distal nodulus and upper tooth shorter and thinner than lower tooth.

Remarks. - *P. amphibiotica* has been recorded from Europe, Africa, China, Indonesia and Japan (Brinkhurst and Jamieson 1971; Ohtaka 2001; Ohtaka and Nishino 2006; Ohtaka *et al*. 2006). This is the first record of the species from Taiwan.

Pristina synclites Stephenson, 1925 (Fig. 3)

Pristina synclites Stephenson, 1925; see Brinkhurst and Jamieson (1971) and Kathman and Brinkhurst (1999) for synonyms and references.

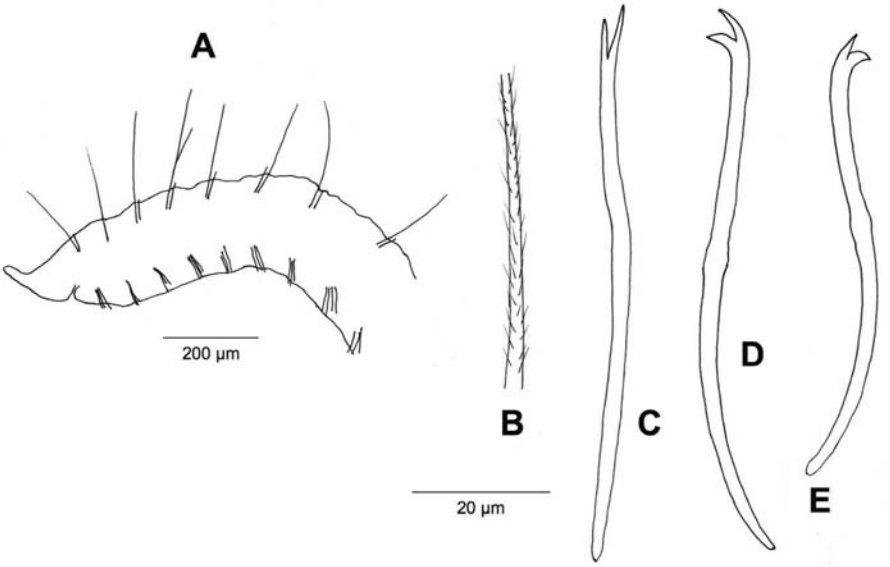

Fig. 3. *Pristina synclites* from Minchien, Nantou Co., Taiwan: A. lateral view of anterior part of body; B. a part of dorsal hair chaeta in an anterior segment; C. dorsal needle chaeta in an anterior segment; D. ventral chaeta in an anterior segment; E. ventral chaeta in a posterior segment.

Material examined. - One immature (ESRI-OA0007) and 20 immature specimens collected from a pond in Minchien, Nantou County (same collection data as for *Dero digitata*).

Brief description. - Body length up to 7 mm long in fixed specimens, forming a chains of zooids. Number of segments in zooids 21-70. Prostomium forming a short proboscis (Fig. 3A). Dorsal hairs 1 or occasionally 2 per bundle, 195-340 µm long, finely hispid except for smooth proximal portion (Fig. 3B). Dorsal needles bayonet-shape, 1-2 per bundle, 64-88 µm long, with weak nodulus and long, bifurcated distal teeth of which upper tooth a little shorter than lower tooth (Fig. 3C). Ventral chaetae in anterior segments 4 or occasionally 5 per bundle, 70-80 µm long, with almost median nodulus (Fig. 3D); those in posterior segments 2-4 per bundle, 64-72 µm long, with distal nodulus (Fig. 3E). Upper tooth in ventral chaetae as long as and thinner than lower tooth in all segments.

Remarks. - *P. synclites* closely resembles *P. americana* Cernosvitov, 1937 by having distinctly hispid dorsal hairs, and the Taiwanese specimens of the former might be assigned to the latter. However, we assigned the specimens to *P. synclites*, because the distal teeth in the ventral chaetae are equally long for all segments (Stephenson 1925; Sperber 1948; Torii and Ohtaka 2007). They differed from *P. americana* by having the upper tooth shorter than lower one in segments after IV (Sperber 1948). Hispid hairs have not been recorded in *P. synclites*, but surface condition of hair chaetae may vary (Chapman and Brinkhurst 1986), and thus, they are not regarded as a reliable character for the species distinction. *P. synclites* was originally described from India (Stephenson 1925) and subsequently recorded from Africa (Brinkhurst and Jamieson 1971), continental China (Wang and Cui 2007), North America (Kathman and Brinkhurst 1999), Kalimantan (Ohtaka *et al*. 2000), Okinawa and Honshu islands, Japan (Torii and Ohtaka 2007) and Taichung County, Taiwan (Lin and Yo 2008a). This species was abundant in the shallow muddy bottom of the farm pond in this study.

Subfamily Tubificinae Vejdovsky, 1876

Limnodrilus hoffmeisteri Claparède, 1862

Limnodrilus hoffmeisteri Claparède, 1862; see Brinkhurst and Jamieson (1971) for synonyms and references.

Material examined. - A mature specimen (ESRI-OA0008) collected from a pond in Minchien, Nantou County (same collection data as for *Dero digitata*).

Remarks. - The specimen of *L. hoffmeisteri* examined had a “plate-topped” penis sheath (Brinkhurst and Jamieson 1971). The species is usually found in eutrophic or organically polluted waters (Ohtaka *et al*. 1990) and is a cosmopolitan and common tubificine in the world (Brinkhurst and Jemieson 1971). It has been repeatedly recorded from Taiwan (Erséus and Hsieh 1997; Hsieh *et al*. 1998, 1999; Lin *et al*. 2005; Lin and Yo 2008a; Lin *et al*. 2008) from heavily polluted estuaries (Erséus and Hsieh 1997) and urban freshwaters (Lin and Yo 2008a).

Aulodrilus japonicus Yamaguchi, 1953

Aulodrilus japonicus Yamaguchi, 1953: 298-300, fig. 12, pl. 7, figs. 5-7.

Material examined. - An immature specimen (ESRI-OA0009) collected from a pond in Minchien, Nantou County (same collection data as for *Dero digitata*).

Brief description. - Body length 9 mm in fixed state. Number of segments 31 with posterior 1 mm being chaetaless. Prostomium short conical. Dorsal chaetal bundles consisted of hairs and crotchets, both beginning on segment II; hair chaetae smooth, 2-9 per bundle, 112-180 μm long; forsal crotchets 5-11 per bundle, 72-107 μm long, with distal nodulus and upper tooth split into several fine teeth which are much thinner and shorter than single lower tooth. Ventral chaetae bifurcated crotchets, 6-11 per bundle, 80-100 μm long, with distal nodulus and upper tooth about half as long as and much thinner than lower tooth. Upper part of the shafts in dorsal and ventral crotchets protruded laterally.

Remarks. - *A. japonicus* resembles *A. pluriseta* (Piguet, 1906) by having dorsal hairs beginning on segment II, no modified genital chaetae, and independent male pores. Brinkhurst Jamieson (1971) once dubiously synonymized the two species. However, *A. japonicus* is distinguishable from *A. pluriseta* by having unique multifold upper teeth in the dorsal crotchets and more posterior position of the midgut, and thus, it should be regarded as a distinct species (Van der Hoek and Verdonschot 2005; Timm and Všivkova 2007). In East Asian *A. japonicus* is widely distributed in Japan (Ohtaka, unpubl.), the Ryukyu islands (Ohtaka 2003), Lake Hanka in Amur basin (Timm and Všivkova 2007), and China mainland (Wang and Cui 2007). Its distributional range may be Holarctic because *A. japonicus* has been often confused with *A. pluriseta* in the Netherlands (Van der Hoek and Verdonschot 2005), Estonia (Timm and Všivkova 2007) and probably in North America (Ohtaka, unpubl.). *A. japonicus* reported herein is the first confirmed record of the species in Taiwan. *A. pluriseta* previously reported by Lin and Yo (2008a) from Taichung County might be *A. japonicus*; the specimens need to be re-examined.

Discussion

Holtodrilus truncatus has recently been reported to live on *Neocaridina* shrimps in Kinki District, Central Japan (Niwa *et al*. 2005). Its occurrence in Japan was thought to be an unintentional introduction along with the importation of the host shrimp for fish bait from China. This area of Japan is famous for leisure fishing and large amounts of *Neocaridina* shrimps are routinely imported (Niwa and Ohtaka 2006). In addition, live alien shrimps are available as “cleaners” for ornamental aquaria via internet sales and from many of local aquarium shops in Japan. The alien shrimps have gradually become widespread (Nishino and Ikeda, unpubl.), leading to a further distribution of symbiotic branchiobdellidans into non-native area. The international trade of shrimps is also popular in Taiwan (Fisheries Agency 2007) and for the same reasons it is probable that *H. truncatus* was introduced to Taiwan along with the importation of its host shrimps.

The introduction of branchiobdellidans on their commercial hosts to Taiwan and Japanese islands is speculative. However, it is equally possible that the branchiobdellidan species tentatively identified as *H. truncatus*, from Miyako Island (Fujita *et al*., unpubl.) is native to the island. This interpretation is supported because its hosts are indigenous *Caridina* shrimps (*C. rubella*, *C. rapaensis* and *C. typus*) in three isolated wells according to Fujita (pers. comm.) and no shrimp are known to have been introduced recently onto the island. This line of reasoning would suggest that *H. truncatus* on shrimps had already become established before the islands of Miyako and Taiwan became separated from the China mainland. No shrimp introduction has been confirmed from outside of the islands. However, Miyako Island is close to Taiwan, and therefore, it is possible that *H. truncatus* is indigenous to Taiwan and closely related to the population on Miyako Island. Further taxonomic and phylogenetic studies of these branchiobdellidans are needed to clarify the origin and biogeographical history of the Taiwanese population.

Incorporating the present records into the past inventory of Taiwanese oligochaetes (Tsai *et al*. 2009), the number of the aquatic microdrile oligochaetes recorded from Taiwan and its adjacent islands increases to 30 species. Erséus and Hsieh (1997) point out that the brackish oligochaete fauna of Taiwan is rather poor, and suggest that the Taiwanese oligochaete fauna is not drastically different from that of continental China (Erséus and Hsieh 1997). However, Taiwan has some unique aquatic environments not encountered in China that need to be explored, such as high mountain ranges with rapid flowing streams and coral reefs facing on the Pacific Ocean. Further studies are required to reveal the full extent of the unique diversity of aquatic oligochaetes on the island.

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附錄、1999-2009年《特有生物研究》期刊目錄索引

第1卷 第1期 (1999年1月)

第2卷 第1期 (2000年5月)

第3卷 第1期 (2001年3月)

第 4 卷 第 1 期 (2002 年 1 月)

第 4 卷 第 2 期 (2002 年 7 月)

第 5 卷 第 1 期 (2003 年 1 月)

第 5 卷 第 2 期 (2003 年 7 月)

第 6 卷 第 1 期 (2004 年 1 月)

第 6 卷 第 2 期 (2004 年 7 月)

第 7 卷 第 1 期 (2005 年 1 月)

第 7 卷 第 2 期 (2005 年 7 月)

第 8 卷 第 1 期 (2006 年 1 月)

第8卷 第2期 (2006年7月)

第9卷 第1期 (2007年1月)

第 9 卷 第 2 期 (2007 年 7 月)

第 10 卷 第 1 期 (2008 年 1 月)

第 10 卷 第 2 期 (2008 年 7 月)

第 11 卷 第 1 期 (2009 年 1 月)

第 11 卷 第 2 期 (2009 年 7 月)

Appendix. Index of“Endemic Species Research”, 1999-2009

Vol. 1, No. 1 (January 1999)

Vol. 2, No. 1 (May 2000)

Occurrence of the Exotic Earthworm *Pontoscolex corethrurus* (Müller) (Glossoscolecidae: Oligochaeta) in Taiwan....................................Chu-Fa Tsai, Huei-Ping Shen and Su-Chen Tsai 68

Vol. 3, No. 1 (March 2001)

Daily Activity Budgets of the Taiwan Ferret Badger (*Melogale moschata subaurantiaca*) in Captivity...Kurtis Jai-Chyi Pei 1

The Occurrence, Distribution and Relative Abundance of Exotic Starlings and Mynas in Taiwan ....Ruey-Shing Lin 13

Using Mitochondrial DNA to Identify Meat Products of the Cervids in Taiwan.......................... ....Yi-Ju Yang, Yao-Sung Lin and Yi-Li Liu 25

Sequences of the 12S rRNA and the Cytochrome b Genes of the Formosan Serow *Naemorhedus swinhoei*... ....Dong-Chi Horng, Shu-Ya Lai, Hsien-Wen Huang, Yu-Chuan Liang and Bor-Rung Ou 37

Cetrarioid Lichens (Parmeliaceae, Ascomycotina) of Taiwan....................................Ming-Jou Lai 49

Cotoneaster subadpressus Yü: A New Record to the Flora of Taiwan....................................... ....Kwo-Shang Lai and Tsung-Hsin Hsieh 67

Feeding Habit of the Formosan Black Bear (*Ursus thibetanus formosanus*) in Captivity............ ....Chieh-Chung Yang, Kuang-Jeng Liaw and Fu-Hsiung Hsu 73

Census Techniques for Bird Surveys..Fu-Hsiung Hsu 81

Vol. 4, No. 1 (January 2002)

Community Structure of Ground-Dwelling Vertebrates Sampled with Drift-Fence Pitfall Traps in a Subtropical Montane Forest in Central Taiwan.. ....Wen-Hao Chou, Ming-Chia Lee and Hon-Tsen Yu 1

The Current Status of Captive Clouded Leopards in Taiwan..........................Lin-Ling Changchien 13

Description of Early Life Stages of the Lycaenid Butterfly *Callenya melaena shonen* (Esaki) (Lepidoptera: Lycaenidae).. ....Lien-Hau Chang, Jen-Shin Hong, Jen-Zon Ho,Wen-I Chou and Jieh Hsiang 35

Analysis of the Earthworm Outbreaks after Chichi Earthquake... ....Guang-Jeng Liaw and Min-Gin Lee 41

Three New Records to Amanitae of Taiwan.....Chien-Ming Chen, Jen-Jye Peng and Tzy-Chau Lin 53

A Remote Sensing Image Analysis for the Tatu Estuary..Tien-Shui Chen 61

The Landscape Pattern Representative Analysis of the Gandaushi Long-Term Ecological Research Site...John-Sin Cheng and Fong-Long Feng 75

Breeding Habit of the Pygmy Wren Babbler *Pnoepyga pusilla* in the Zueyenshi Major Wildlife Habitat in Central Taiwan............................Cheng-Te Yao, Tai-Lin Ai and Hsiow-Chen Huang 87

A Partial Albino of the Formosan Leaf-Nosed Bat *Hipposideros terasensis* Kishida from

Vol. 4, No. 2 (July 2002)

Vol. 5, No. 1 (January 2003)

Vol. 5, No. 2 (July 2003)

Biodiversity of the True Slime Molds (Myxomycetes) at Mt. Shamao of the Yangmingshan National Park..Jong-How Chang, Ing-Gia Huang and Chin-Hui Liu 1

Habitat Assessment of Creeks for the Propagation of *Oncorhynchus masou formosanus* (Jordan & Oshima) in Taiwan.....................................Ming-Fon Yeh, Shi-Tsang Chang and S-Jeng Lin 15

Distribution of Exotic Freshwater Fishes and Shrimps in Taiwan.. ....Ruong-Tzung Chen, Ping- Ho Ho and Hsun-Hwang Lee 33

Bird Assemblages in Relation to Vegetation Recovery at Mt. Jeou-jeou-fen of Taiwan after the 921 Earthquake................Scott Ruey-Shing Lin, Fu-Hsiung Hsu, Cheng-Te Yao and Tai-Lin Ai 47

Remote Sensing Analyses for the Land-cover Changes in the Coastal Zone of the Choshui Estuary..Tien-Shui Chen 61

Notes on the Mindoro Snake Eel *Lamnostoma mindorum* (Jordan and Richardson) (Ophichthidae: Pisces) in the Western Pacific........................Shi-Tsang Chang and Chu-Fa Tsai 73

A New Species of the Earthworm Belonging to the Genus *Metaphire* Sims and Easton (Megascolecidae: Oligochaeta) from the Northeastern Taiwan.. ....Chu-Fa Tsai, Jiun-Hong Chen, Su-Chen Tsai and Huei-Ping Shen 83

A New Record of the Bithecal Megascolecid Earthworm *Amynthas papilio* (Gates) (Oligochaeta) from Taiwan...Jiun-Hong Chen and Shu-Chun Chuang 89

Vol. 6, No. 1 (January 2004)

Biodiversity of Sea Urchins in the Maoaw Bay of the Northeastern Taiwan............................... ....Kao-Sung Chen and Ying-Chou Lee 1

Conservation Genetics of *Zacco pachycephalus* by Microsatellite DNA Locus Analyses............ ....Mei-Tzu Huang, Eric Hsien-Shao Tsao and Hon-Tsen Yu 17

Egg and Larval Developments of the Taiyal Darner *Aeshna petalura taiyal* Asahina (Odonata, Aeshnidae) at the Mt. Hohuan...Sue-Cheng Lin and Chin-Seng Chen 29

Altitudinal Differences in Temporal Distribution, Spatial Preference and Timing of Breeding Climax of Frogs and Toads in the Central Taiwan.................Chun-Fu Lin and Kuang-Yang Lue 39

The Mindoro Snake Eel *Lamnostoma mindorum* (Jordan and Richardson) of the Hsiukuluan River in the Eastern Taiwan..Shi-Tsang Chang and Chu-Fa Tsai 51

A Morphological Study on Spores of 10 Species of Dennstaedtiaceae from Taiwan with Scanning Electron Microscopy Technique... ....Lee-Yu Kuo, Wei-Lung Wang, Chi-Lin Wang and Min-Ya Sheng 57

Ambrosia psilostachya DC. (Asteraceae) a Newly Naturalized Plant in Taiwan.......................... ....Yen-Hsueh Tseng and Ching-I Peng 71

Vol. 6, No. 2 (July 2004)

Vol. 7, No. 1 (January 2005)

Vol. 7, No. 2 (July 2005)

Vol. 8, No. 1 (January 2006)

Vol. 8, No. 2 (July 2006)

Seasonal Variation in Altitudinal Distribution of Plumbeous Water Redstart (*Rhyacornis fuliginosus affinis*) in Taiwan......................Mei-Ru Su, Shi-Tsang Chang and Ruey-Shing Lin 1

A Risk Assessment System of Exotic Terrestrial Vertebrates for Taiwan...Meng-Wen Fan, Ruey-Shing Lin, Ya-Lun Huang and Te-En Lin 7

Artificial Breeding and Larval Development of the Taiwan Lesser-Bream *Rasborinus formosae* (Oshima, 1920)...Hong-Thih Lai, Jui-Tsung Chang and Jhy-Yun Shy 23

A New Species of the Gnaphosid Spider Belonging to the Genus *Aphantaulax* (Araneae: Gnaphosidae) from Taiwan...Yih-Tsong Ueng and Tzu-Yang Weng 31

Temporal and Spatial Variations of Epilithic Periphyta in the Upper Tachia River of the Wulin Area..Shu-Fen Yu, Hsing-Juh Lin and Pei-Chung Chen 39

Vegetation Classification System of the *Machilus-Castanopsis* Zone in the Central-West Climatic Region of Taiwan........................Ching-Yu Liou, Horng-Jye Su and Yen-Hsueh Tseng 53

Landscape Dynamics of the Lutao Island of Taiwan...............Yuh-Lurng Chung and Ming-Lun Lu 87

A New Record of *Haptosquilla glyptocerus* (Wood-Mason, 1875) (Stomatopoda: Protosquillidae) from Taiwan...Jung-Fu Huang and Pan-Wen Hsueh 97

Ipomoea eriocarpa R. Br. (Convolvulaceae) Newly Naturalized to Taiwan..Tsai-Wen Hsu, Tzen-Yuh Chiang, Kun-Chan Tsai and Chao-Ching Huang 103

Vol. 9, No. 1 (January 2007)

Genetic Patterns and Biogeographic Origins of the Exotic Rodent (*Rattus exulans*) in Taiwan.... Jui-Hua Chu,Yu-Jen Tsao, Yuan-Ju Chuang, Huang-Chi Chen, Yao-Sung Lin and Hai-Yin Wu 1

The Diet of Tawny Owls (*Strix aluco*) Analyzed by Their Pellets on Roadsides in Mountain Areas of the Central Taiwan...............................Wen-Loung Lin, Yin Wang and Hui-Yun Tseng 13

Comparison of Breeding Ecology of Zitting Cisticola (*Cisticola juncidis*) and Tawny-Flanked Prinia (*Prinia subflava*) in the Rice Paddy............................Liu-Chih Lo and Feng-Mao Cheng 23

Butterfly Fauna and Nectar Plants at the Chinghsien Ecological Park, Taichung City, Central Taiwan...Dah-Wei Li 37

Vesicular-arbuscular Mycorrhizal Fungi (VAMF) symbiotic with Pioneer Plants at Mt. Jiujiufong after the 921 Earthquakes..Tzy-Chau Lin and Chi-Guang Wu 51

A Comparison of Three Agamic Methods on Rooting of *Magnolia kachirachirai* Seedling Plants ....Kun-Yun Hong, Zong-Fang Lee and Fuh-Jiunn Pan 63

A New Record of the Octochaetine Earthworm *Dichogaster saliens* (Beddard, 1892) from the Centro-western Taiwan..Huei-Ping Shen and Chu-Fa Tsai 71

Basilicum polystachyon (L.) Moench a Rare Mint in Taiwan.............................Tsung-Hsin Hsieh 75

Vol. 9, No. 2 (July 2007)

Vol. 10, No. 1 (January 2008)

Vol. 10, No. 2 (July 2008)

Vol. 11, No. 1 (January 2009)

Vol. 11, No. 2 (July 2009)

《台灣生物多樣性研究》稿約

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   Soule, M. E. and B. A. Wilco. 1980. Conservation biology: An evolutionary-ecological approach. Sinauer Associates, Sunderand, Massachusetts.

   Jinchu, H. and W. Fuwen. 1990. Development and progress of breeding and rearing giant pandas in captivity within China. pp. 322-325. *In*: H. Jinchu (ed). Research and progress in biology of the giant panda. Sichuan Publishing House of Science and Technology, Sichuan, China.
8. Table should be typed on a separate sheet and be headed by a title of dual languages (Chinese and English). It consists of only horizontal lines and typed with English terms (if possible) and Arabic numerals. If foot notes are required, mark with superscripts ^{1}, ^{2}, *, $^{\#}$, etc.
9. Figure should be drawn with black ink on a separate white tracing paper with a figure legend of the dual languages below. Computer graph made from laser printer is acceptable.
10. Photograph should be a glossy black and white shot with sufficient resolution to be clearly legible after reduction. When multiple photos are employed, the author should arrange them in plates. Micrographs should include bars indicating scales of magnification. Photos should be pasted on white A4 paper loosely with the figure legend below.