TAXONOMIC RE-EVALUATION OF CHLORITIS BIFOVEATA (BENSON 1856) AND C. DIPLOCHONE MÖLLENDORFF 1898 (PULMONATA: CAMAENIDAE)

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Abstract Morphological comparison of the two congeneric allopatric camaenid land snails, Chloritis bifoveata (Benson 1856) and C. diplochone Möllendorff 1898, was evaluated. Both species show strong similarities in some unique characters such as the extraordinary biconcave shell shape. However, the species differ in shell size with C. bifoveata the smaller of the two, and also in length of the penis and vagina, again shorter in C. bifoveata. The penis and vagina are of almost equal length and size in C. bifoveata are almost equal but in C. diplochone the penis is longer and more slender than the vagina. The distributions of the species are also very different with C. bifoveata occurring in southern Myanmar, Thailand and northern Malaysia and C. diplochone in eastern Thailand to the Indochina region.

Key words Chloritis, Camaenidae, land snail, Gastropoda, Thailand

INTRODUCTION

Chloritis Beck 1837, the largest genus of the Camaenidae, consists of eight subgenera (Zilch, 1960) with over 150 nominal species (Richardson, 1985). Identification of the known species has proved to be difficult (Pilsbry, 1891; Godwin-Austen, 1920) due to the diversification and high similarity of shell morphology amongst species. Therefore, taxonomic clarification based only on shell characters is insufficient. Although several authors have highlighted this problem and suggested that the genital anatomy should be elucidated to solve the problem (e.g. Pilsbry, 1891, 1894; Godwin-Austen, 1920), an anatomical review has not been published despite the reproductive anatomy of a few species being known (e.g. Pilsbry, 1894; Moss & Webb, 1896; Rensch, 1933, 1937).

From the 20 or so *Chloritis* species known from Thailand, Laos and Cambodia, *C. bifoveata* (Benson 1856) and *C. diplochone* Möllendorff 1898, with their peculiar biconcave shell shape have been largely overlooked and there are no intensive studies (Gude, 1903, 1906; Panha, 1996). According to the current available information, the distribution of *C. bifoveata* is confined to the Malay Peninsula (Henley & Theobald, 1876; Hemmen & Hemmen, 2001), whilst *C. diplochone* is distributed in Indochina (Möllendorff, 1898; Panha & Thanamitramanee, 1997). Although, Möllendorff 1898 stated that *C. diplochone* has a similar shell morphology to *C. bifoveata*, with only the shell size being different, more than a hundred years later there is still no reported informative characters to discriminate between these two potentially allopatric species. Recently, we have collected snails of both species from various localities in Thailand. These specimens provided an opportunity for anatomical examination to look for new characters including their reproductive anatomy.

The aim of the present study is to conduct a morphological evaluation of *C. diplochone* and *C. bifoveata* including genitalia, radula, jaw and shell micro-sculptures.

MATERIALS AND METHODS

Both shell and live material were collected from eastern and southern Thailand and peninsular Malaysia. Snails were drowned in water and then fixed in 70% v/v ethanol for anatomical examination. Specimens were identified using Benson (1856) and Möllendorff (1898), and later compared with type specimens. For anatomical purposes, three specimens were dissected and the drawing of one specimen was selected as representative of the species. The adults were measured for shell height (h) and shell width (d). The radular formula and teeth shape, embryonic shells and periostracal sculptures were examined under a SEM (JEOL, JSM-5410).

In the description of genitalia, proximal refers to the region closest to the genital orifice and

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distal to the region furthest away from the genital orifice. The anatomical abbreviations used are defined by Pilsbry (1894), Sutcharit and Panha (2006), and Sutcharit *et al.* (2007).

Institutional abbreviations CUMZ, Chulalongkorn University, Museum of Zoology, Bangkok, Thailand; SMF, Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, a.m.

Systematic Description

Family CAMAENIDAE Albers 1850 Genus *Chloritis* Beck 1837

Type species Helix ungulina Linnaeus 1758; subsequent designation by Martens in Albers (1860).

Diagnosis Shell low discoidal, flattened to concave, small to large and thick to thin, with five or more whorls; umbilicus narrow to wide. Surface smooth or with pits, nodules or granulation arranged along growth lines. Periostracum transparent to moderately thick, brownish and fibrous or with hairs in oblique rows. Aperture rounded, oblique or securiform. Peristome whitish to brownish; lip expanded and slightly reflected. Genitalia without dart apparatus; penial verge present; penis connected to epiphallus, with short to long flagellum. Radula with triangular and tricuspid teeth. Jaw brownish corneous with strong vertical ridges.

Distribution A widely distributed genus ranging from southern China to Japan, Indochina, Sundaic islands, northern part of Australia, New Guinea and New Zealand (Solem, 1959; Zilch, 1960; Richardson, 1985; Schileyko, 2003).

Remarks The genus *Chloritis* comprises 8 subgenera (Zilch, 1960; Vaught, 1989), of which subgenus *Trichochloritis* is distributed in Indochina, Sundalands and the Philippines (Pilsbry, 1894; Schileyko, 2007). *Trichochloritis* as originally nominated included some 40 nominal species (Pilsbry, 1891, 1894). However, the subgeneric position of the species considered here were mainly based on shell characters and geographical distribution (*sensu* Pilsbry, 1891, 1894; Zilch, 1960). The distinguishing characters of the subgenus are a wide umbilicus and the presence of a truncated ridge around the umbilicus (Pilsbry, 1891, 1894; Schileyko, 2007). However, Schileyko (2007) raised *Trichochloritis* to generic level, a change that may have to be re-considered when the anatomy of the type species, *"Helix breviseta* Pfeiffer, 1862", is available for examination and comparison.

> *Chloritis bifoveata* (Benson 1856) Figs 1A, B, 2A–F, 3A-D; Table 1.

Helix bifoveata Benson 1856: 251; Type locality: Therabuin, Tenasserim, Burma. Henley & Theobald, 1876: 7, pl. 14, fig. 8. Helix (Chloritis) bifoveata Benson – Pilsbry, 1891: 245, 246; pl. 50, figure between figure 36 and 42. Chloritis bifoveata (Benson) – Pilsbry, 1891: 320 (figure legend). Richardson, 1985: 87. Hemmen & Hemmen, 2001: 46, fig. 17. Maassen, 2001: 120. Chloritis (Trichachloritic) hifowata (Bonson)

Chloritis (Trichochloritis) bifoveata (Benson) – Pilsbry, 1894: 124. Gude, 1906: 116. Gude, 1914: 167.

Material examined Wat Khao Kok, Kanchnadit, Suratthani: CUMZ 3531; Wat Sathitkhirirom, Khiriratnikhom, Suratthani: CUMZ 4658, 4677; Tam Ka-min, Nasarn, Suratthani: CUMZ 4682; Tam Pannara, Nakornsrithammarat: CUMZ 3533; Khao Poo Chaow, Trang: CUMZ 3510; Khao Huay Haeng, Huay Yod, Trang: CUMZ 3527, 3528; Botanic Garden, Trang: CUMZ 3605, 3607, 3611; Khao Chong, Trang: CUMZ 3501, 4666; Ton Nga Chang Waterfall Wildlife Sanctuary, Songkhla: CUMZ 3786, 4680; Tao Thong Waterfall, Tubpud, Pangnga: CUMZ 3529, 3788, 4672; Tam Nampud, Tubpud, Pangnga: CUMZ 3530, 3532, 3787, 4181; Wat Khiriwong (Tam Koop), Tubpud, Pangnga: CUMZ 3525; 3645; 3789, 4671; Khao Oak Talu, Patthalung: CUMZ 3540, 3601, 3612, 3646; Khao Poo-Khao Ya National Park, Patthalung: CUMZ 3608, 3792; Koh Bu Loanpai, Krabi: 3603; Sra Morakot, Krabi: CUMZ 4662, 4667, 4673, 4676, 4678; Wat Tam Sue, Krabi: CUMZ 3526, 3790, 3791, 4657, 4664, 4665, 4668, 4670, 4674, 4675; Tam Srikesorn, Ratthaphum, Songkhla: CUMZ 4663; Kuan Karong, Satun: CUMZ 4659; La Ngu, Satun: CUMZ 4661; Tarutao National Park, Satun: CUMZ 4660; Bukit Chuping, Peris, Malaysia: CUMZ 3636, 4185; Langawi Island, Peris, Malaysia: CUMZ 3635, 3637.



Figure 1 Shells and live snails characteristics. *Chloritis bifoveata* from Songkhla (CUMZ 3786) (A), and from Krabi (CUMZ 3789) with shell width approximately 15 mm (B). *Chloritis diplochone* from Plieu Waterfall (CUMZ 3647) (C), and from Makok Waterfall (CUMZ 3785) with shell width approximately 20 mm (D). White arrows indicate the shell constriction.

Description Shell rather small, thin, dextral, brownish to dark brown in colour, biconcave in shape (dorsoventrally concave) (Fig. 1A, B). Embryonic shell smooth; following whorls with corneous to brownish periostracum, short hairs arranged in an oblique row along the growth line, and transversely micro ridged (Fig. 2A–C). Whorls 5 to 6 well-rounded, increasing regularly; spire concave, resembling an umbilicus. Descent of last whorl beginning about ¹/₄ whorl from aperture, and constriction occurring at about ¹/₂ whorl from aperture i.e. opposite aperture. Aperture subvertical, narrowly sickle- or crescent-shaped. Peristome brownish; lip expanded

a little and slightly thickened. Umbilicus wide, but narrower than the concave upper side and shouldered.

Radula and jaw Teeth arranged in almost straight rows. Each row with 73 (35–1–37) teeth. Central tooth triangular tricuspid with pointed mesocone and very small ectocones. Lateral teeth tricuspid, mesocone with sharp cusp; ectocone and endocone small (Fig. 2D). Latero-marginal teeth moderately long and oblique tricuspid-shaped; endocone placed near tip of the tooth; ectocone moderately large and located in the middle level of the tooth (Fig. 2E). Outermost teeth short;

Table 1	Shell measurements and whorls count of <i>Chloritis bifoveata</i> (Benson 1856) and <i>Chloritis diplochone</i>				
Möllendorff 1898. The number in parentheses are CUMZ catalogue numbers.					

Species, Locality and	Number	Ranges, Mean ± SD in mm of:			Whorl
CUMZ nos.		Shell Height	Shell Width	h/d Ratio	
Chloritis bifoveata					
Botanic Garden Trang	1 -	8.7–9.8	13.9–16.5	0.57-0.62	51/8-57/8
(3605, 3607, 3609, 3611)	15	9.13 ± 0.35	15.37 ± 0.69	0.59 ± 0.02	
Bukit Chuping, Perlis,	9	8.5-9.5	13.7-16.6	0.56-0.62	5-5%
Malaysia (3636, 4185)		9.05 ± 0.41	15.24 ± 0.77	0.59 ± 0.02	
Tam Nam Pud, Pangnga	15	8.1–9.8	13.3-15.7	0.58-0.69	5-5%
(3530, 3532, 3787)		9.02 ± 0.45	14.64 ± 0.72	0.62 ± 0.03	
Nat Tam Keep, Pangnga	29	7.8–9.9	13.2-16.1	0.57-0.65	51/8-56/8
(3525, 3789)		8.09 ± 0.53	14.62 ± 0.80	0.61 ± 0.02	
Wat Khao Huay Haeng,	18	7.5-8.9	12.1-15.1	0.54-0.63	5 ³ / ₈ -5 ⁶ / ₈
Krabi (3527, 3528)		8.00 ± 0.40	13.85 ± 0.80	0.58 ± 0.02	
Wat Tam Sue, Krabi (3526,	54	7.7–10.1	12.7–16.6	0.58-0.66	5 ⁴ / ₈ -6 ¹ / ₈
3790, 3791, 4155)	94	9.11 ± 0.51	14.93 ± 0.83	0.61 ± 0.02	
Ton Nga Chang W.S.,	7	8.0-8.7	13.2–14.7	0.59-0.61	54/8-6
Songkhla (3786)	7	8.32 ± 0.25	13.98 ± 0.54	0.59 ± 0.01	
Chloritis diplochone					
Makok W.F., Chanthaburi	-	9.3-10.9	17.0-19.4	0.53-0.60	5%-64/8
(2620, 3614, 3647, 3785)	7	10.11 ± 0.57	18.13 ± 0.85	0.56 ± 0.02	
Trong Nong W.F., Chanthaburi	9	8.3–9.9	15.9–18.3	0.52-0.59	$5^{2}/_{8}-6^{4}/_{8}$
(3602, 3606)		9.36 ± 0.45	17.37 ± 0.95	0.54 ± 0.02	

mesocone, endocone and ectocone similar in size with pointed cusps (Fig. 2F).

Jaw thickened, corneous-brownish, crescentshaped, anteriorly convex with cutting margin. Vertical ribs strong and prominent and variable in number (Fig. 3C).

Genitalia Atrium (at) very short. Penis (p) moderately short but broad; epiphallus (e) longer than penis; flagellum (fl) short. Penial retractor muscle (pr) thin and relatively long. Vas deferens (vd) a small tube, connected between free oviduct and end of epiphallus. Internal wall of penis ribbed with series of swollen and smooth longitudinal pilasters (pp), and line introverted to penial chamber to encircle the irregular-shaped penial verge (pv) (Fig. 3A).

Vagina (v) stout, short and about half of penis length. Gametolytic duct (gd) proximally enlarged but suddenly tapering to a small tube; distally connected to swollen gametolytic sac (gs). Free oviduct (fo) small; oviduct (ov) enlarged towards alveoli; prostate gland situated under oviduct. Albumen gland (ag) thin with a long tongue shape. Hermaphroditic gland (hg) with somewhat numerous lobules. Hermaphroditic duct (hd) a small, twisted tube connected close to head of talon (ta). Internal wall of vagina with several longitudinal vaginal pilasters (vp) with swollen and smooth pilaster surface (Fig. 3B).

Living snail The living animal has blackish-grey reticulated skin; dorsally with lighter stripe starting from between posterior tentacles and along dorsal part of body length. Foot pale brown to greyish, posterior tail rather wide with scattered light mottle. Posterior tentacles are drumstick-shaped and distally light brown; anterior tentacles and mouth part are greyish (Fig. 1B). Headwart small and curved, located between posterior tentacles (Fig. 3D). Mantle edge and mantle cavity are blackish.

Distribution Chloritis bifoveata was originally reported from the village of Therabuin, Tenasserim or Burma. No subsequent specimens and localities were added for more than 150 years until Hemmen and Hemmen (2001) reported specimens from southern Thailand. The recent collections are known in several localities in southern peninsular Thailand, recorded south of the type locality and ranging from Suratthani, Nakornsrithammarat,

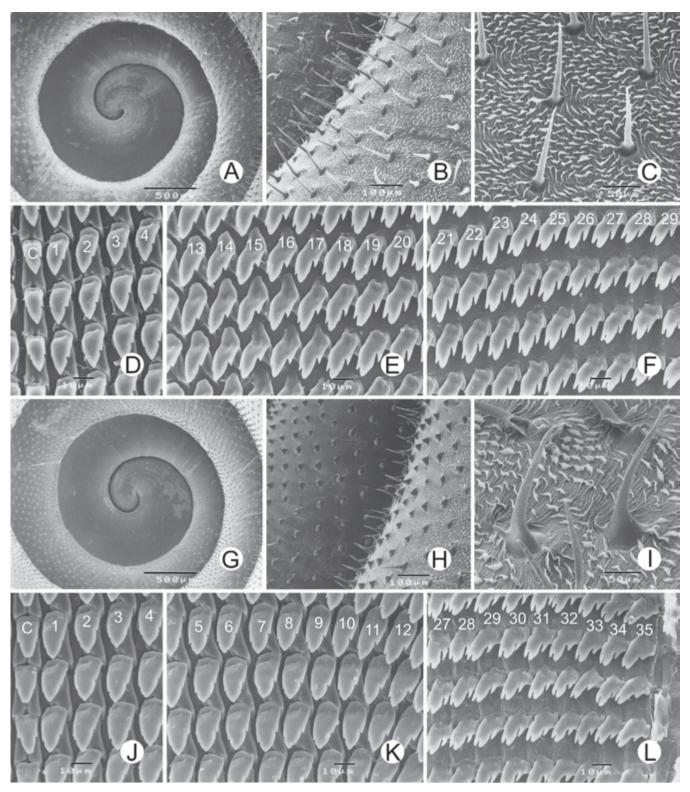


Figure 2 SEM images of protoconch, periostracal structure and radula. *Chloritis bifoveata* from Patthalung (CUMZ 3646) (A–F), and *Chloritis diplochone* from Chanthaburi (CUMZ 3647) (G–L). (A, G) protoconch sculpture. Periostracal structure: (B, H) periostraca with rows of periostracal hairs; (C, I) hairs and micro-sculptures on periostraca. Radula morphology: (D, J) central teeth with the first to the fourth lateral teeth; (E, K) lateral teeth with the transitional tricuspid marginal teeth; (F, L) marginal teeth. Central tooth indicated by 'C'. Numbers indicate the tooth order from lateral to marginal end.

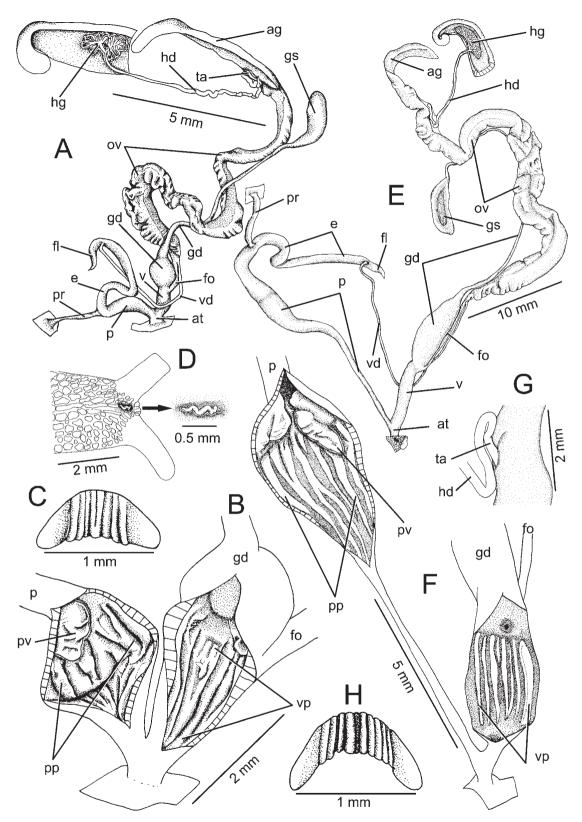


Figure 3 Genital organs and jaws of *Chloritis bifoveata* from Patthalung (CUMZ 3608) (A–C), and *Chloritis diplochone* from Chanthaburi (CUMZ 3615) (D–F). (A, E) general characteristic of genital system; (B, F) interior structures of penis, atrium and vagina chamber; (C, H) jaws; (D) dorsal view of head portion showing headwart. Abbreviations: ag, albumen gland; at, atrium; e, epiphallus; fl, flagellum; fo, free oviduct; gd, gametolytic duct; gs, gametolytic sac; hd, hermaphroditic duct; hg, hermaphroditic gland; ov, oviduct; p, penis; pp, penial pilaster; pr, penial retractor muscle; pv, penial verge; v, vagina; vd, vas deferens; vp, vaginal pilaster.

Chloritis diplochone Möllendorff 1898 Figs 1C, D, 2G–L, 3E–H; Table 1.

Chloritis diplochone Möllendorff 1898: 72; Type locality: Boloven. Richardson, 1985: 94. *Chloritis (Trichochloritis) diplochone* Möllendorff – Gude, 1906: 116. Zilch, 1966: 301, pl. 7, fig. 8 (Lectotype SMF 8594). Panha, 1996: 36. Panha & Thanamitramanee, 1997: 2, fig. a.

Material examined Lectotype SMF 8594, Paralectotype SMF 8595 (1 shell). Trong Nong Waterfall, Plieu National Park, Chanthaburi: CUMZ 3602, 3606, 3613. Wat Khao Chakan, Srakeo: CUMZ 3609. Khao Soi Dao, Chanthaburi: CUMZ 3610. Makok Waterfall, Chanthaburi: CUMZ 3614, 3615, 3647, 3648, 3785, 3993, 4679, 4681.

Description Shell morphology is similar to the previous species. The major differences are larger shell size, whitish apertural lip and slightly longer periostracal hairs (Figs 1C, D, 2G–I).

Radula and jaw Each row contained about 69 (34–1–34) teeth. Central tooth tricuspid, dulled mesocone and very small ectocones (Fig. 2J, K). Latero-marginal (Fig. 2K), outermost teeth (Fig. 2L) and jaw (Fig. 3H) are similar to previous species.

Genitalia Penis longer and more distinct in penial sculptures than *C. bifoveata*. Atrium (at) very short. Penis (p) extremely long, somewhat slender; proximally very thin; distally a little enlarged. Internal wall of penis ribbed by series of thin longitudinal pilasters (pp). In the distal part pilasters line the introverted penial chamber and encircle penial verge. Penial verge (pv) small and cone shaped (Fig. 3E, F).

Female genital organ similar to the previous species. Only slightly distinct in that the internal wall of vagina possesses several thin longitudinal vaginal pilasters (vp) with smooth pilaster surface (Fig. 3G, F).

Living animal The living snail is blackish grey. Others characters are similar to the previous species. *Distribution Chloritis diplochone* was known from Boloven Plateau in southern Laos (Möllendorff, 1898) and a hundred years later from eastern Thailand (Panha & Thanamitramanee, 1997). The recent collections were recorded from several localities in Chanthaburi and Srakeo Provinces.

DISCUSSION

Both species described here exhibit a unique biconcave shell with brownish and hairy periostracum, but where the periostracum has disappeared only a hair-base or nodule scars remain. Although, C. bifoveata and C. diplochone have a similar shell shape and periostracal structures, distinct differences have been observed. Chloritis diplochone has a larger shell size (Table 1), and longer penis and epiphallus, whilst C. bifoveata has a smaller shell size (Table 1), with a penis of similar length to the vagina, and the proximal part of the gametolytic duct swollen with a rounded shape (see also description and figures). These characteristics provide sufficient evidence for the two entities to be considered separate species. They are allopatric with each having fairly clearly delimited ranges which do not overlap. Chloritis bifoveata has been found in southern Thailand and northern peninsular Malaysia, while C. diplochone is known only from eastern Thailand and possibly Laos (Möllendorff, 1898).

Only two species of the subgenus Trichochloritis out of a total of ten Chloritis s.l. species have had their genitalia and radula examined (Wiegmann, 1893; Moss & Webb, 1896; Pilsbry, 1905; Rensch, 1933, 1937; Schileyko, 2007). The absence of a pouch-like enlargement of the penis found in other species, distinguishes both C. bifoveata and C. diplochone, from for example C. crassula (Philippi 1845) (Wiegmann, 1893). The long epiphallus, short flagellum and penial verge provide good characters to distinguish C. brevidens (Sowerby 1841) which lacks these (Schileyko, 2007). The camaenid radula with a triangular unicuspid central tooth, tricuspid lateral and marginal teeth and ribbed jaw is found in the two species described here and across the genus (see also Pilsbry, 1894; Moss & Webb, 1896; Pilsbry, 1905; Rensch, 1933, 1937). However, supplementary genital anatomy and radular morphology from the type species and other congener species is needed to clarify and improve the systematics of the *Chloritis* (*Trichochloritis*).

In our survey, snails were found around heavy, decayed logs or tree stumps in moist evergreen forests where they appeared to feed on various fungal species. This may be a temporary condition, but in our long experience of collecting the snails have never been found in other habitats. The snails may possibly live or aestivate in soil, waiting for fungi to fructify on decomposing fallen trees. In tropical evergreen forests like those of Thailand the log decomposition occurs throughout the year. So what appear as temporary habitats may have more permanence. More surveys are needed to clarify this point.

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