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Phuphania costata, a new species of dyakiid land snail (Pulmonata: Dyakiidae) from Phu Pha Lom limestone area, Loei Province, northeastern Thailand

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Abstract. *Phuphania costata* sp. nov. is described from Phu Pha Lom limestone area, Loei Province, Northeastern Thailand. Morphological features were examined and compared with *Phuphania globosa* Tumpeesuwan, Naggs & Panha 2007 and other species belonging to related genera. It is hypothesized that a decrease in the number of clusters of the internal ducts in the amatorial organ gland, and a thickening of the sac of the amatorial organ gland in *P. costata* are derived characters in the genus *Phuphania*. The geographically disjunct distributions of *P. globosa* and *P. costata* may be a result of dry climatic conditions during the Pleistocene, leading to allopatric speciation in *Phuphania.*

Key words. Dyakiidae, *Phuphania*, anatomy, disjunct distribution, northeastern Thailand

INTRODUCTION

The family Dyakiidae is a poorly known family of the Limacoidea sensu lato (Baker, 1941), which has to date received little attention by malacologists. Laidlaw (1931) was the first researcher to list the dyakiid genera and he also defined the family in morphological terms. The duct of the gametolytic sac connecting to the base of the amatorial organ is a unique characteristic of Dyakiidae.

In Thailand, five genera belonging to family Dyakiidae were reported, including *Dyakia* Godwin-Austen, 1891, *Quantula* Baker, 1941, *Pseudoplecta* Laidlaw, 1932, *Bertia* Ancey, 1887, and *Phuphania* Tumpeesuwan, Naggs & Panha, 2007 (Panha, 1996; Hemmen & Hemmen, 2001; Nabhitabhata, 2009). The phylogenetic relationships among these dyakiid land snails have not received much attention, except for the cladistic analysis of Hausdorf (1995) who described the relationships among the related genera, in which *Everretia* exhibited the most derived character state. However, Tumpeesuwan et al. (2007) more recently suggested that members of the genus *Bertia* have a more derived amatorial organ than *Everretia*, and that *Phuphania* species displayed the most derived state in the Dyakiidae.

Phuphania globosa Tumpeesuwan, Naggs & Panha, 2007 is a land snail endemic to the Phu Phan Range in northeastern Thailand (Fig. 1). This species is the sole member of the recently proposed genus *Phuphania* Tumpeesuwan, Naggs & Panha, 2007 in the family Dyakiidae (Gude & Woodward, 1921). The diagnostic characters of *Phuphania* include the presence of small ducts clustered within the amatorial organ gland, and fused lobes forming a cap over the amatorial organ (Tumpeesuwan et al., 2007).

Between October 2011 and October 2012, land snails were collected from Phu Pha Lom Limestone in Loei Province, Northeastern Thailand (Fig. 1). One species with a dextral and depressed shell exhibited a genital system with the gametolytic duct running from the base of the amatorial organ, a synapomorphy of the Dyakiidae. Further examination showed that it belongs to the genus *Phuphania*, but distinct from *P. globosa.*

MATERIAL AND METHODS

The snails were collected from the ground and under leaf litter between October 2011 to October 2012 in mixed deciduous and dry evergreen forest at Phu Pha Lom Limestone Hill (17°33'86.1"N, 101°52'35.9"E), Loei Province, northeastern Thailand. Fourteen specimens were drowned in water and preserved in 70% ethanol prior to examining their radula and reproductive systems. These specimens were deposited in the Natural History Museum, Mahasarakham University (NHMSU). Intact adult shells (n=66) were used to determine whorl number, and shell height (h) and shell width (w) were measured using Vernier calipers. Radulae were extracted from five adult snails and examined under a scanning electron microscope (JEOL, JSM-5410LV). Radula shape and teeth formula were observed and recorded. Seven adult snails were also dissected to examine the reproductive system.

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Type material was deposited in the following institutions: Natural History Museum, Mahasarakham University, Maha Sarakham, Thailand (NHMSU); The Zoological Reference Collection (ZRC), Mollusc Section, Raffles Museum of Biodiversity Research, National University of Singapore; Natural History Museum of Loei Rajabhat University (NMLRU); and Zoological Research Collection of Burapha University (ZRCBUU).

Abbreviation. In the description of the genital system and external morphology, we used the term "proximal" to refer to the position closest to the genital opening, and "distal" to refer to the position furthest from the genital opening. The terminology and abbreviations used here follows Tumpeesuwan et al. (2007) and Sutcharit et al. (2012): ag: albumen gland; am: amatorial organ; amg: amatorial organ gland; at: atrium; camg: cluster of internal duct of amatorial organ gland; cf: caudal foss; ch: caudal horn; ep: epiphallus; fo: free oviduct; gs: gametolytic sac; hd: hermaphroditic duct; p: penis; pg: prostate gland; pr: penial retractor muscle; ut: uterus; v: vagina; and vd: vas deferens.

SYSTEMATICS

Family Dyakiidae Gude & Woodward, 1921

Subfamily Dyakiinae Gude & Woodward, 1921

Genus *Phuphania* Tumpeesuwan, Panha & Naggs, 2007

Type species. *Phuphania globosa* Tumpeesuwan, Panha & Naggs, 2007

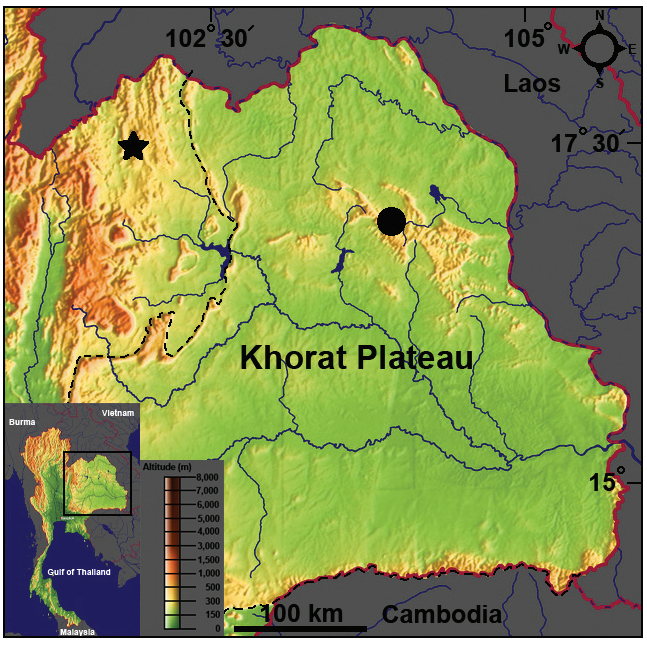

Fig. 1. Type locality of *Phuphania costata*, new species, Phu Pha Lom, Mueang District, Loei Province (star); type locality of *Phuphania globosa* (circle) on Phu Phan Range. Dashed line indicates the approximate boundary between Khorat Plateau and other parts of Thailand. (modified from GinkgoMaps−project; http://www.ginkgomaps.com)

***Phuphania costata*, new species**
Figs. 2–5; Table 1

Phuphania sp.: Tanmuangpak et al., 2012: 18, 20, 22–24

Type material. Holotype: NHMSU-0002 (Fig. 2). Measurements: shell height 20.7 mm, shell width 29.9 mm; type locality: Phu Pha Lom Limestone Mountain, Loei Province, northeastern Thailand. coll. K. Tanmuangpak, October 2012. Paratype: NHMSU-0003 (63 shells); ZRC MOL (1 shell); NMLRU-0001 (1 shell); ZRCBUU 03395 (1 shell), coll. K. Tanmuangpak, October 2011–October 2012.

Etymology. "*costata*" from the dense and regularly radial ribs on the dorsal part of the shell surface.

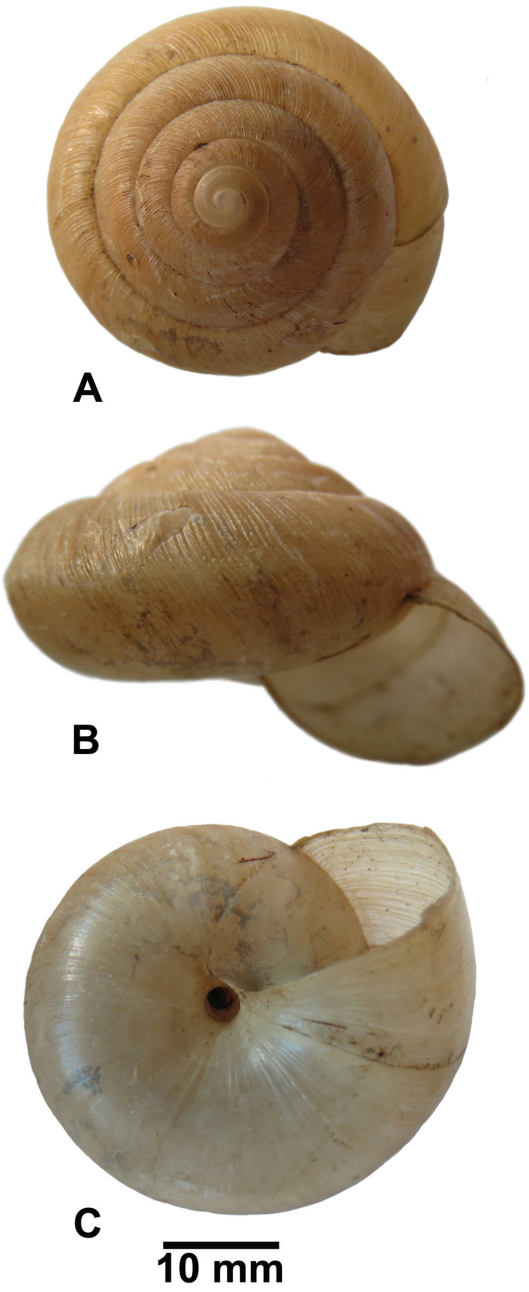

Fig. 2. *Phuphania costata*, new species, Phu Pha Lom Limestone Mountain, Loei Province. Holotype (NHMSU-0002): A, dorsal view; B, apertural view; C, ventral view.

Table 1. Comparison of shell, genital system, and radula of *Phuphania* species.

Character	*Phuphania globosa* (Tumpeesuwan et al., 2007)	*Phuphania costata*, new species
Shell	Semiglobose-globose peripheral keel absent fine radial growth lines	Semidepressed-depressed peripheral keel present distinct radial ribs
Vas deferens	Short, entering epiphallus subapically	Long, entering epiphallus apically
Epiphallus	Slender and elongate	Swollen
Penis	Clavate	Short cylindrical
Vagina	Outer surface with longitudinal rugose sculpture	Outer surface smooth
Gland of amatorial organ	Ovate The lobes fuse as very thin sac Consists of 5–6 clusters of internal ducts	Crescent-shaped The lobes fuse as very thick sac Consists of 2–4 clusters of internal ducts
Radula: marginal teeth	Short, finger shaped with blunt tip	Elongate, slender finger shaped with sharp pointed tip

Diagnosis. The new species possesses 2–4 clusters of internal ducts within the amatorial organ gland, fused lobes forming very thick sac over the amatorial organ, and the vas deferens enters the epiphallus apically (Fig. 4; Table 1). Dorsal shell surface possesses dense and regularly radial ribs.

Description. Shell: Dextral, sub-depressed, height 14.9–20.7 mm, shell width 22.7–29.9 mm, 5¾ convex whorls, slightly thin, body whorl large, slightly angulated at periphery, fulvous above, embryonic whorls with fine radial ribs, subsequent whorls with distinct radial ribs, 28–31 ribs per 10 mm (Fig. 2A, B). Aperture slightly large, aperture height 10.2–13.1 mm, aperture width 12.4–16.1 mm, crescent shape, distinctly oblique, lip neither expanded nor reflected. Umbilicus narrow.

Body: Ground colour of body grey, marked with black spots. Foot sole undivided, caudal horn without overhanging lobe, caudal foss with short vertical slit in tail (Fig. 3).

Genital system: Atrium (at) very short, nearly absent. Penis (p) short cylindrical. Epiphallus (ep) proximal part clavate, proximal end more swollen than distal end and penis, distal end equal in size to distal part. Penial retractor muscle (pr) slightly swollen at the middle, inserts near distal end of epiphallus.Vas deferens (vd) narrow, longer than penis and vagina (v), entering epiphallus apically. Amatorial organ (am) large, stoutly cylindrical sac. Amatorial organ gland (amg) forms thick crescent shaped cap connect to the amatorial organ and consists of 2–4 clusters of internal duct of amatorial organ gland (camg). Free oviduct (fo) rather short. Large and elongate gametolytic sac (gs) connects to the base of the amatorial organ, which possesses an apical ligament that attaches to the prostate gland (pg) (Figs. 4A–D).

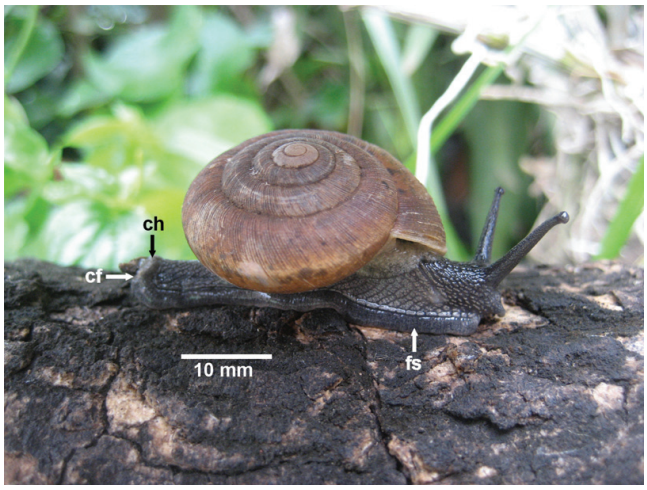

Fig. 3. *Phuphania costata* new species. Phu Pha Lom Limestone Mountain, Loei Province. Paratype (NHMSU-0003).

Radula: Each row of teeth is arranged in a wide angled V formation, where the central tooth is lanceolate and tricuspid. Lateral teeth blade-shaped, tricuspid with tiny lateral cusps, endocone closer to the tip of the central cusp than ectocone (Fig. 5A–C). Size and shape of teeth gradually changes to slim finger shape and endocone absent to form bicuspid at the last 7^{th}–8^{th} teeth from the outermost lateral teeth (Fig. 5D, E). Marginal teeth 12–20, unicuspid, narrow, elongated and finger-shaped (Fig. 5F, G).

DISCUSSION

Phuphania costata, differs from the type species of the genus *Phuphania*, *P. globosa*, by its shell shape, shell sculpture, genital system, and radula. Conchologically, *P. costata* has a more angulate peripheral keel and distinct radial ribs compared to *P. globosa* (Table 1). Liew et al. (2009) concluded that shell surface sculpture is a consistent characteristic within species of *Everettia*, another genus of the family Dyakiidae. The shell of *P. costata* also closely resembles the common Siamese land snail, *Cryptozona siamensis* (Ariophantidae), but the new species differs in having a slightly angulated periphery, with its subsequent whorls having distinct radial ribs and lacking a dark brownish color dorsally. Their genitalia are also significantly different from each other. The genital system of *P. costata* has the same characters as *P. globosa*, with regards to the absence of a common amatorial organ duct, small ducts clustered within the amatorial organ gland, and major lobes of the amatorial organ gland fused to form a cap covering the amatorial organ. These characters are probably synapomorphies for the genus *Phuphania*. The amatorial organ gland of *Phuphania* species is distinct from those in the other dyakiid genera, in having its fused lobes form a cap covering the tip of the organ. This

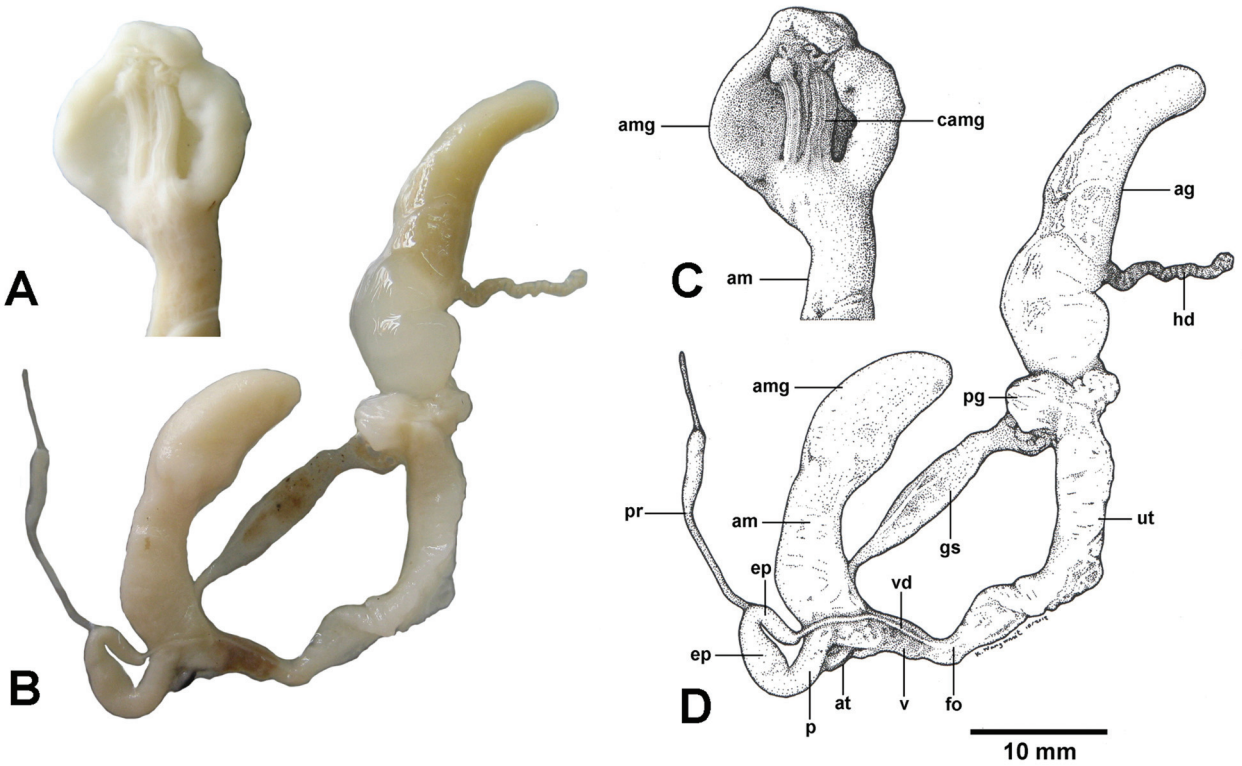

Fig. 4. *Phuphania costata*, new species. Genital system of paratype (NHMSU-0003): A, internal structure of amatorial organ gland (amg); B, genital system; C, schematic drawing of internal structure of amatorial organ gland (amg); D, genital system. (scale bar provided applies A through D).

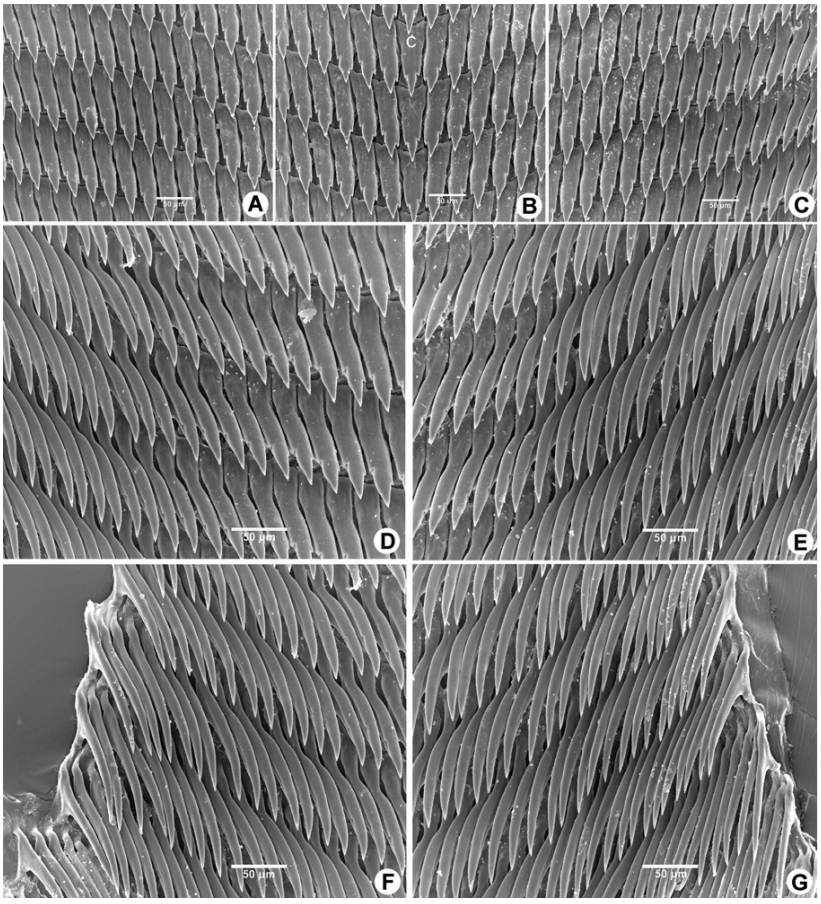

Fig. 5. *Phuphania costata*, new species. Radula of paratype (NHMSU-0003): A, left lateral teeth; B, central teeth (indicated by letter 'C') and lateral teeth; C, right lateral teeth; D–E, transitional from tricuspid to bicuspid lateral teeth and unicuspid marginal teeth on both left and right side of central teeth; F, left marginal teeth; G, right marginal teeth.

cap consists of clusters of internal small ducts entering the amatorial organ apically. There are 2–4 clusters in *P. costata*, in contrast to 5–6 clusters in *P. globosa*. The gametolytic duct is inserted at the base of the amatorial organ, which is similar to that found in members of the genus *Bertia* but it is shorter in *Phuphania* species. The gametolytic sac of *P. costata* is also larger compared to *Bertia* (Schileyko, 2003), with a terminal ligament attached to the prostate gland that is absent in *Bertia* species.

The phylogeny of Dyakiidae is largely based on a transformation series of increasing complexity and number of glands and ducts supplying the amatorial organ (Hausdorf, 1995). The condition of one or two glands connected by a single duct, such as in *Quantula* was hypothesized to be a plesiomorphic condition. Increasing complexity in gland and duct numbers were assumed to be a condition of derived states, for which *Everettia* exhibited the most derived character state in Hausdorf's cladogram. Tumpeesuwan et al. (2007) used an extension of Hausdorf's transformation series. They proposed that the amatorial organ system of *Bertia* (Schileyko, 2003) is more derived than that of *Everettia*, and *Phuphania* possessed the most derived state in the Dyakiidae, with clusters of numerous internal ducts supplying glandular secretions to the amatorial organ. The decrease in the number of clusters of internal ducts in the amatorial organ gland and thickening of the sac at the tip of the amatorial organ of *P. costata* might be conceived as derived characters in *Phuphania*. We therefore propose here that *P. costata* possesses the most derived state in Dyakiidae.

The new species, *P. costata* is distributed in the limestone area of Phu Pha Lom, Loei Province, which is situated westward of the Mesozoic sandstone escarpment of the Khorat Plateau. It is a long way from the distribution range of *P. globosa* in the Phu Phan Mountain Range approximately 250 km away. Such a disjunct distribution within *Phuphania* might indicate allopatric speciation.

The geographical isolation of the small population at the western rim of the parent population of *Phuphania* could lead to allopatric speciation following the uplifting of the Khorat Plateau. This occurred after the collision of the Indian subcontinent with Eurasia during the middle to the end of the Eocene (45–35 Ma) (Metcalfe, 2011). The uplifting of the plateau raised the escarpment and mesa (Bunopas et al., 2003), which may have gradually separated the western and eastern populations of *Phuphania* from each other due to the elevation of the escarpment at the western rim of the Khorat Plateau.

During the Pleistocene, the Khorat Plateau changed in elevation and inclination, whereas central Thailand and the Cambodian Plain subsided. This would have allowed time for *Phuphania* to become adapted to the unique dry climatic pattern. *Phuphania globosa* became established in the Phu Phan mountain range on the Khorat Plateau, whereas, *P. costata* became established in the limestone area outside the Khorat Plateau. The disjunct distribution on the flat area of the Khorat Plateau might be caused by a dry period during the Pleistocene. During this time, it seems conceivable that the flat areas were affected by climatic change, leading to the local extinction of *Phuphania* and other land snails there.

There is some evidence based on the composition of fauna and flora of the Pleistocene Epoch of East Asia and Southeast Asia to support this hypothesis. In the case of the giant panda *Ailuropoda melanoleuca*, its recent distribution is restricted to high mountainous areas (2,700–3,900 m above mean sea level) in southern China, which are covered with temperate forest. The undergrowth is mainly temperate bamboo species such as *Sinarundinaria fangiana* and *Fargesia spathacea* (Tougard et al. 1996), which are consumed by pandas. According to their recent distribution, these two bamboo species grow only in areas where the mean temperature is about 0°C in winter and 16°C in summer, and where the mean annual rainfall is about 1,100 mm (Reid et al., 1989).

A fossilised tooth of *Ailuropoda melanoleuca baconi* was discovered in a cave deposit (Latest Middle Pleistocene) in Chaiyaphum Province, which is strongly correlated to the distribution of two species of bamboo, *S. fangiana* and *F. spathacea* (Tougard et al. 1996). In the Pleistocene Epoch, a drastic climatic change occurred in Southeast Asia (van der Kaars & Dam, 1995), and during the last interglacial it seemed less favorable for temperate bamboo in Thailand. This climatic condition may have caused the geographical limits of temperate bamboo species to collapse northwards and consequently, so did the range of the giant panda. The retreat in the distribution of the range of the giant panda was caused by the retreat of the temperate bamboo northward to the cooler area. This situation might be similar to that involving *Phuphania*. The disappearance of the damp forest and suitable habitats in the flat area may be due to climatic change. The phylogenetic relationships among the species in the genus and the period of speciation will be elucidated in future studies.

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