

Karyotype Variations in the Genus *Pollicaria* (Caenogastropoda: Pupinidae)

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Bangon Kongim, Chirasak Sutcharit, Piyoros Tongkerd, Shau-Hwai Aileen Tan, Nguyen Xuan Quynh, Fred Naggs, and Somsak Panha (2010) Karyotype variations in the genus *Pollicaria* (Caenogastropoda: Pupinidae). *Zoological Studies* 49(1): 125-131. The karyotypes of 4 recognized Southeast Asian species of the genus *Pollicaria* were examined. All 7 populations studied were found to possess the same chromosome pattern: $n = 13$, $2n = 26$. However, the fundamental chromosome number (FN) of both populations of *P. elephas* from the south (Malaysia) was 46, while those for the other 3 species (5 populations) were all 50. Moreover, although each species had a unique karyotype, the karyotype of the isolated species, *P. elephas*, differed from the other 3 northern species in exhibiting 3 distinct telocentric chromosomes ($2m+6sm+2st+3t$). The 3 smaller species, *P. mouhoti* from northeastern Thailand, *P. myersii* from Laos, and *P. grandidata* from 2 localities in northern Vietnam, exhibited similar karyotypes of $6m+4sm+2st+1t$, $4m+6sm+2st+1t$, and $(3m+7sm+2st+1t$ and $2m+8sm+2st+1t)$ respectively, with only 1 telocentric chromosome. The karyotypes of the 2 morphologically variant populations of *P. grandidata* from Vietnam differed by 1 in the number of metacentric and submetacentric chromosomes. The genus *Pollicaria* exhibits slight rearrangements in the centromeric positions of the smaller chromosomes and the deletion of 1 chromosome pair. A ZZ-ZW sex-determining system is proposed for this genus. <http://zoolstud.sinica.edu.tw/Journals/49.1/125.pdf>

Key words: Operculate snails, *Pollicaria*, Karyotype variation, Sex determination, Sex chromosome.

The operculate land-snail genus *Pollicaria* Gould, 1856 is endemic to threatened limestone outcrops of Southeast Asia (Kobelt 1902, Gude 1921, Solem 1966, Pain 1974, Abbott 1989), where species occur in highly localized populations. The snails of this genus are often called elephant pupinids or elephant snails because of their gigantic size relative to other members of the family Pupinidae. The form and color of the shell are highly distinctive; the shell is large and inflated, has an expanded aperture in the adult, and is orange. Although the isolated, southern species,

P. elephas, which is the largest of the species, has a cream-colored shell, the aperture and peristome of the shell are bright orange (Morgan 1885, Pain 1974). The distributional range of *Pollicaria* is quite narrow, with at least 4 species from the genus having been recorded in the northern part of Southeast Asia (Myanmar, Thailand, Cambodia, Laos, and Vietnam) and only 1 species, *P. elephas*, recorded in the south, on the Malay Peninsula (Morgan 1885, Kobelt 1902, Pain 1974). So far, the 4 recognized species of *Pollicaria* have been entirely discriminated based on shell characters

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(Crosse 1885, Fischer 1885, Pain 1974). Even the recent work by Pain (1974), a revision of the systematics of *Pollicaria*, was based solely upon shell morphology, with *P. gravida* from Vietnam being hypothesized to have 2 shell morphotypes (i.e., a large, brown-shelled form and a smaller, orange form, Fig. 1). In this study, we karyologically analyzed both morphotypes of *P. gravida* using samples of the smaller form collected from Cuc Phuong National Park and of the large form from Khe Se in northern Vietnam. We compared these forms with 3 other *Pollicaria* species. Our initial anatomical investigation of *Pollicaria* indicated that the 2 morphotypes of *P. gravida* were conspecific, as suggested by Pain (1974), but the karyotypic analysis presented here suggests that the 2 forms are distinct species.

The karyotypes of the 4 species of *Pollicaria* from Thailand, Laos, Vietnam, and Malaysia, presented herein, represent the 1st karyotypic analysis of the genus *Pollicaria*.

MATERIALS AND METHODS

The locality, sex (i.e., determined by the

presence/absence of a penis near the lower right tentacle), and number of snails karyotyped are given in figures 1, 2 and table 1. Species identifications were made on the basis of published work (Gould 1856, Benson 1856 1859, Haines 1858, Pfeiffer 1862, Crosse 1885, Fischer 1885, Morgan 1885, Mabille 1887, Kobelt 1902, Gude 1921, Habe 1964, Solem 1966, Pain 1974), and by comparison with type material at the Natural History Museum, London, UK (NHM), Muséum National d'Histoire Naturelle, Paris, France (NMHN) and Senckenberg Museum, Frankfurt, Germany (SMF).

Chromosome preparations were made from metaphase-arrested gonadal tissue of both males and females using an air-drying method modified from Patterson and Burch (1978) and Park (1994). Gonads were enriched for mitotic metaphase cells by the direct injection with 0.1 ml of 0.1% colchicine (Sigma, D-89552 Steinheim, Germany). After a period of 4 h, the gonads were dissected and cut into small pieces after being placed in a 0.07% hypotonic KCl solution. The separated cells were collected by centrifugation at 1000 rpm for 10 min, and were subsequently fixed in fresh Carnoy's fixative (3 parts absolute ethanol with



Fig. 1. Shells of (a) *Pollicaria mouhoti*, (b) *P. myersii*, (c) *P. gravida* (big brown shell), (d) *P. gravida* (small orange shell), and (e) *P. elephas*.

1 part glacial acetic acid). The supernatant was replaced with fresh fixative for each of 2 centrifugation-based washes. The cells were re-suspended, and the suspensions were placed

on clean glass slides, which had been preheated to 60°C. The slides were air-dried and then stained with a 4% Giemsa solution for 15 min. Photomicrographs of 10 well-spread metaphase

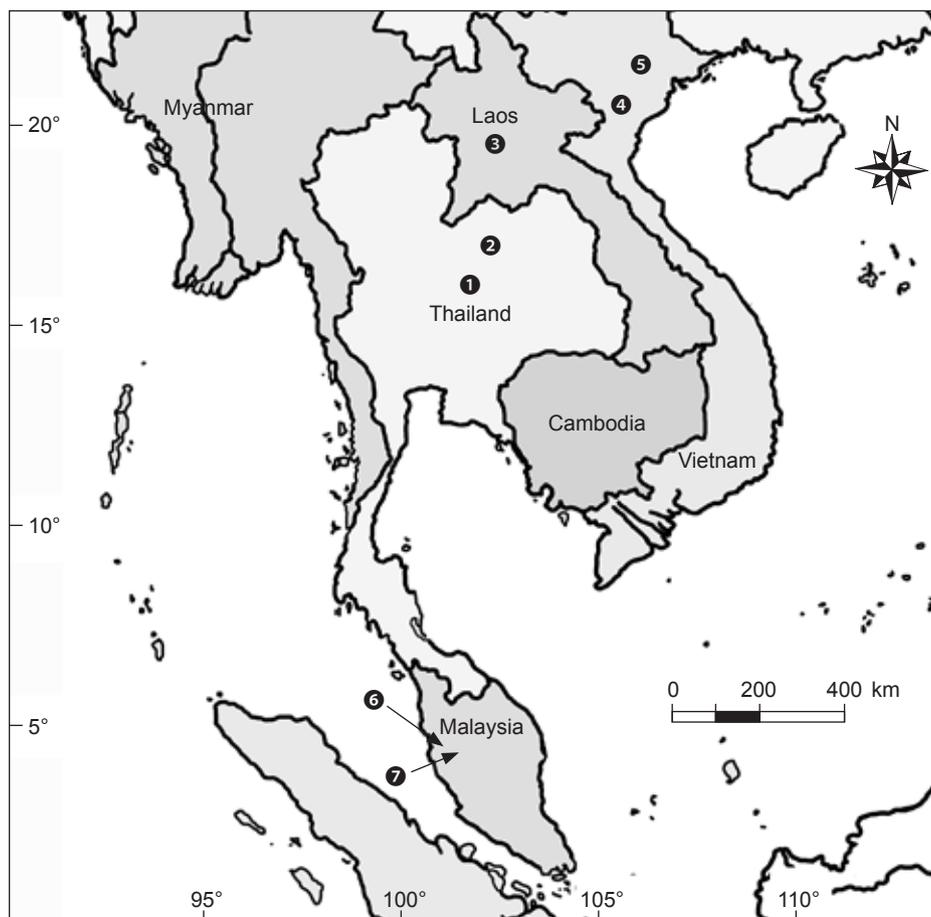


Fig. 2. Southeast Asian localities where *Pollicaria* was sampled in this study. Collection locality numbers are the same as those in table 1.

Table 1. Scientific names, sample localities, diploid number ($2n$), fundamental number (FN), and karyotype formula of species of *Pollicaria* investigated in the present study

| Scientific name | Locality | Sex | No. | ($2n$, FN) | Karyotype |
|--|--|--------------|--------|--------------|---------------|
| <i>P. mouhoti</i> (Pfeiffer, 1862) | 1. Chiayaphum, Thailand | Male, Female | 10, 9 | 26, 50 | 6m+4sm+2st+1t |
| <i>P. mouhoti</i> (Pfeiffer, 1862) | 2. Khon Kaen, Thailand | Male, Female | 9, 7 | 26, 50 | 6m+4sm+2st+1t |
| <i>P. myersii</i> (Haines, 1858) | 3. Vang Vieng, Laos | Male, Female | 10, 6 | 26, 50 | 4m+6sm+2st+1t |
| <i>P. gravida</i> (Benson, 1856) (small orange shell) | 4. Cuc Phuong, Ninh Binh, Vietnam | Male | 10 | 26, 50 | 2m+8sm+2st+1t |
| <i>P. gravida</i> (Benson, 1856) (big brown shell) | 5. Khe Se, Vietnam | Male | 10 | 26, 50 | 3m+7sm+2st+1t |
| <i>P. elephas</i> (Morgan, 1885) | 6. Tangung Rambutan, Ipoh, Perak, Malaysia | Male, Female | 10, 10 | 26, 46 | 2m+6sm+2st+3t |
| <i>P. elephas</i> (Morgan, 1885) | 7. Gunung Genting, Ipoh, Perak, Malaysia | Male, Female | 10, 10 | 26, 46 | 2m+6sm+2st+3t |

m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric chromosome.

cells were measured to evaluate the relative length and centromeric index. Mitotic karyotype images were arranged and numbered for chromosome pairs in order of the decreasing mean relative length; the nomenclature of morphological chromosome types followed that of Levan et al. (1964).

RESULTS

The 4 species (from 7 populations) of *Pollicaria* all exhibited the same number of haploid and diploid chromosomes ($n = 13$, $2n = 26$), but the 2 fundamental chromosome numbers (FNs) were 46 in both *P. elephas* populations, compared to 50 in the other 3 species.

Each species possessed a unique karyotype, but the isolated Malaysian species, *P. elephas*, significantly differed from the other 3 northern species. It exhibited 3 distinct telocentric chromosomes ($2m+6sm+2st+3t$), in contrast to the single telomeric chromosome of the 3 northern species. The karyotypes of *P. mouhoti* from northeastern Thailand, *P. myersii* from Laos, and *P. grvida* from 2 localities in northern Vietnam were more similar to each other than to that of *P. elephas* and were respectively $6m+4sm+2st+1t$, $4m+6sm+2st+1t$, and ($3m+7sm+2st+1t$ and $2m+8sm+2st+1t$) (Figs. 3, 4, Table 1). The number of metacentric and submetacentric chromosomes of the 2 morphologically variant populations of *P. grvida* from Vietnam (Fig. 1, Table 1) differed by 1.

Comparison of the female karyotypes of the 4 species, *P. mouhoti* from Chiayaphum, Thailand, *P. myesii* from Vang Vieng, Laos, *P. elephas* from Ipoh, Malaysia, and *P. grvida* from Cuc Phuong, Vietnam, revealed distinct size heteromorphism of metacentric and submetacentric chromosome pairs akin to ZW chromosomes (Fig. 4). In contrast, all of the males examined had homologous pairs of chromosomes. This suggests the presence of ZZ-ZW sex chromosomes (ZZ in males and ZW in females) and a probable sex-determining system.

The 3 northern species showed a larger portion of metacentric chromosomes than telocentric chromosomes with a ratio of 10: 3. In contrast, the southern species, *P. elephas*, has a ratio of 8: 5, and this evidence indicates that chromosome rearrangements have occurred in *Pollicaria*. The form, aperture, and color of the shell of *P. elephas* greatly differed from those of the other 3 species (Fig. 1).

DISCUSSION

Karyotype data offer valuable systematic characters in cyclophoroideans because the metaphase chromosomes in the group exhibit a clear bi-armed form which is rarely found in land pulmonate snails (Burch 1967, Kongim 2006). Chromosome numbers in the superfamily Cyclophoroidea are highly conserved. For example, the very common genus *Cyclophorus* shows a relatively invariable low diploid number ($2n = 28$), the presence of 2 distinct size groups, and the numerical predominance of metacentrics in both size groups, while some species contain

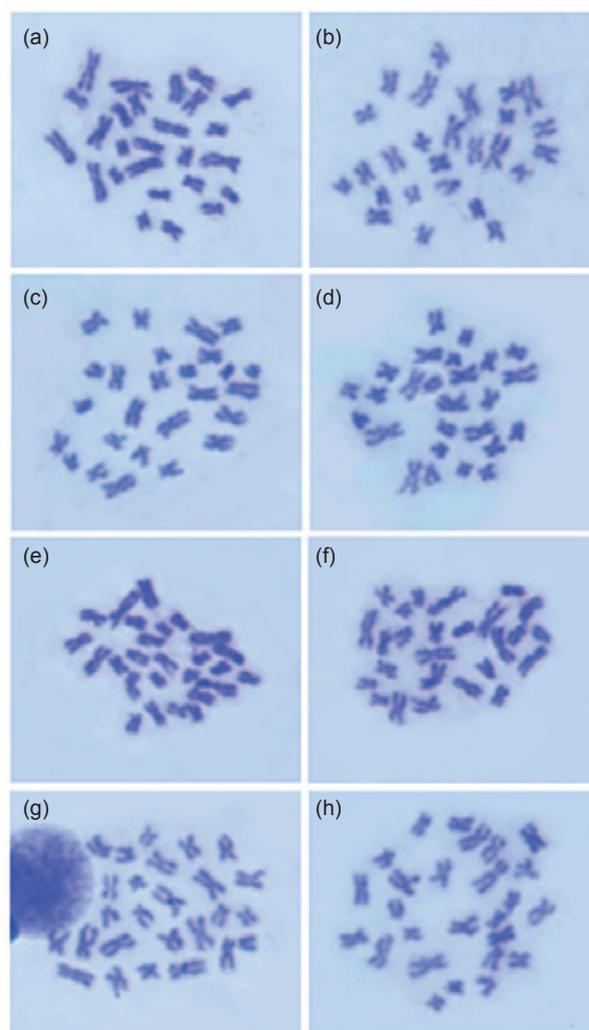


Fig. 3. Mitotic chromosome spreads of the 4 recognized Southeast Asian *Pollicaria* species: (a) *Pollicaria mouhoti* (male); (b) *P. mouhoti* (female); (c) *P. myersii* (male); (d) *P. myersii* (female); (e) *P. elephas* (male); (f) *P. elephas* (female); (g) *P. grvida* (male of big brown shell); and (h) *P. grvida* (male of small orange shell).

only metacentric chromosomes (Kasinathan and Natarajan 1975, Choudhury and Pandit 1997, Kongim et al. 2006). The 10 tiny cyclophoroideans belonging to *Diplommatina* also show a conservative chromosome number similar to that of *Pollicaria* ($2n = 26$) and similar morphologies of the metacentric and submetacentric chromosomes (Ieyama and Tada 1991, Ieyama et al. 1993, Ogaito and Ieyama 1997, Ieyama and Ogaito 1998, 2000). Also within the Pupinidae, Burch (1967) demonstrated the presence of haploid ($n = 13$) and diploid chromosome numbers ($2n = 26$) in *Pupina* sp.

Karyotypes of *Pollicaria* described in this study indicate a divergence in characteristics of both karyotype formation and the lesser FN value between *P. elephas*, and the 3 other species analyzed. This result is consistent with the current geographic distribution of these taxa. *Pollicaria elephas* is the only species recorded from the south, whereas the other 3 species have been recorded from more-northerly parts of Southeast Asia.

In contrast to *P. elephas* which has a telocentric karyotype, the 3 northern species have karyotypes that share many similarities with each other, such as metacentricity mostly in pairs 1 to 9, and an FN value of 50. According to White (1978a), karyotypes with a higher proportion of metacentric

chromosomes are probably primitive, and show relative stability, a notion that is supported by the degree of asymmetry in karyotypes where the primitive karyotype has the lowest asymmetry and the derived karyotype has higher asymmetry (Diupotex-Chong et al. 2004). Thus, in showing the highest asymmetry, with $2m+6sm+2st+3t$, the karyotype of *P. elephas* is hypothesized as exhibiting a derived character state within *Pollicaria*, whereas the karyotype with the lowest asymmetry in *Pollicaria*, exhibited by *P. mouhoti* (Thailand) with $6m+4sm+2st+1t$, is hypothesized to be a plesiomorphic state.

As is currently known, *P. gravida* ranges in distribution from Myanmar (type locality) to Vietnam and is notable for exhibiting significant variation in shell morphology. Nevertheless, our examination of shells from various natural history museums in Europe showed that the shell characters described above are a consistent feature of this species. It is, therefore, noteworthy that comparisons of the karyotypes of populations of *P. gravida* from Khe Se and Cuc Phuong, northern Vietnam differed (i.e., $3m+7sm+2st+1t$ and $2m+8sm+2st+1t$, respectively), as did the shell morphology, particularly the shell size and color of the periostracum. These differences are consistent with King's (1993) suggestion that the 2 populations are distinct species. Karyotypic

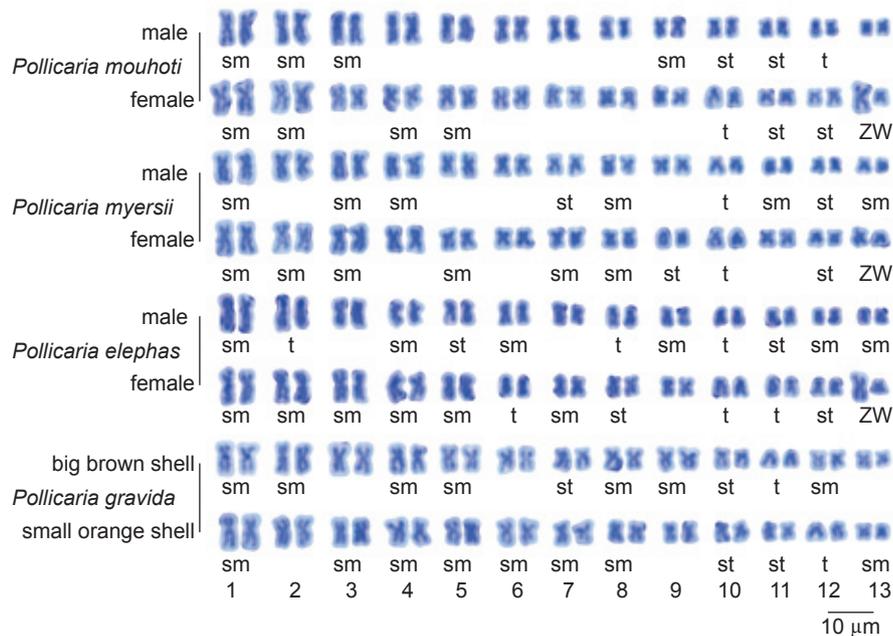


Fig. 4. Karyotypes of the 4 recognized Southeast Asian *Pollicaria* species: *P. mouhoti* (male above and female below); *P. myersii* (male above and female below); *P. elephas* (male above and female below); *P. gravida* (big brown shell above and small orange shell below). Metacentric (unlabeled); sm, submetacentric; st, subtelocentric; t, telocentric.

divergence may occur as part of population differentiation, as well as an adaptive component in phyletic evolution (Bickham and Baker 1979, Bush et al. 1977, White 1973 1978b, Espinosa et al. 2007). Such chromosomal differences are often associated with species-level differentiation and may in some cases either cause or reinforce speciation events (Swanson et al. 1967, Patton and Sherwood 1983, Clark and Wall 1996, Rickart et al. 1999)

The ZZ/ZW-type sex chromosome determination is known to occur in *Cyclophorus* (Caenogastropoda: Cyclophoridae) (Kongim et al. 2006) and other related gastropods (Baršienė and Ribi 1998–1999, Baršienė et al. 2000). The likely existence of ZZ/ZW sex chromosomes and thus a sex-determining system in *Pollicaria* is not conclusive given the rapid and multiple evolution of different sex-determining systems including ZZ/ZW as exhibited between other closely related animals such as in the Lepidoptera (Yoshido et al. 2005, Traut et al. 2008).

As with other cyclophoroideans, species boundaries between allopatric populations are extremely difficult to determine. For example, an anatomical study of *P. elephas* by Fischer (1885) and an investigation including the 'soft' anatomy (unpublished investigation by BK) found that the 3 species of *P. elephas* from Ipoh, Malaysia, *P. myersii* from Laos, and *P. mouhoti* from Thailand, showed no significant differences. With current evidence, morphological information alone provides insufficiently distinct characters to allow recognition of species within the Cyclophoroidea (Kongim 2006, Lee et al. 2008). Karyotype analysis in combination with anatomical information and karyotype analysis is proving to be a valuable tool for determining species-level boundaries and relationships in terrestrial operculate snails.

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