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Karyotype Differentiation within the Elephant Pupinid Snail, *Pollicaria mouhoti* (Pfeiffer, 1862) (Caenogastropoda: Pupinidae)

BANGON KONGIM[1], CHIRASAK SUTCHARIT[2], PIYOROS TONGKERD[2] AND SOMSAK PANHA[2*]

[1]Department of Biology, Faculty of Science, Mahasarakham University, Kantharawichai District, Maha Sarakham, 44150, Thailand.
[2]Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.

ABSTRACT.– Mitotic chromosomes of *Pollicaria mouhoti* were analyzed from gonad tissue. The diploid chromosome number is n = 13, $2n$ = 26, the fundamental chromosome number is FN = 50. However, intraspecific variation was found between populations of *P. mouhoti* located in different regions of Thailand. Specimens of the northern dark form possess karyotypes of (7m+3sm+2st+1t) with five large and seven median chromosomes, whereas the southern typical forms are (6m+4sm+2st+1t) with nine large, three medium and one small chromosome. Likewise, differences in the relative length (RL) and centromeric index (CI) between these two allopatric morphs were noted. The existence of heteromorphic chromosome pairs in females but not males supports a potential ZZ (male) – ZW (female) sex chromosome system, as reported for other species in this genus. The dark form specimens, from Loei and Nongbualamphu Provinces, exhibited smaller shells which were black in color throughout their shell, while the typical form specimens from Chaiyaphum, Khon Kaen, Phetchabun and Phitsanulok Provinces had larger shells with a brown body and a whorl which was orange colored on the three last whorls. The results of this study are consistent with the two allopatric morphological forms being distinct subspecies.

KEY WORDS: Pupinidae, Taxonomy, *Pollicaria mouhoti*, Karyotype differentiation

INTRODUCTION

The elephant pupinid snails, *Pollicaria* Gould, 1856, are endemic to threatened limestone outcrops, and they live in highly localized populations. Currently, five nominal species are recognized and their known distributions are quite narrow, ranging from Burma to Thailand, Cambodia, Laos, Vietnam and the Malaysian Peninsula (Kobelt, 1902; Pain, 1974). In Thailand, only a single species, *Pollicaria mouhoti* (Pfeiffer, 1862), has been recorded. It can be recognized by its pupa shape, a small to large shell and a monochrome blackish or yellowish to bright orange shell color (Kobelt, 1902; Pfeiffer, 1862). They are important decomposers and they live under the leaf litter.

* Corresponding author:
Tel: (662)-218-5273
e-mail:b.kongim@msu.ac.th

Land snail surveys, carried out throughout Thailand since 1996, have recorded two allopatric different shell forms within this species. They are a 'typical form' with an orange color on the upper spire (Fig. 1A) and relatively large shells, and a 'dark form' with smaller monochrome blackish shells (Fig. 1B) (Kongim and Panha, unpub. data). Given this distinct morphology and allopatric distribution, it is plausible that they represent distinct subspecies or even recent new species, but with so few informative or fast evolving characters this requires a genetic approach for confirmation.

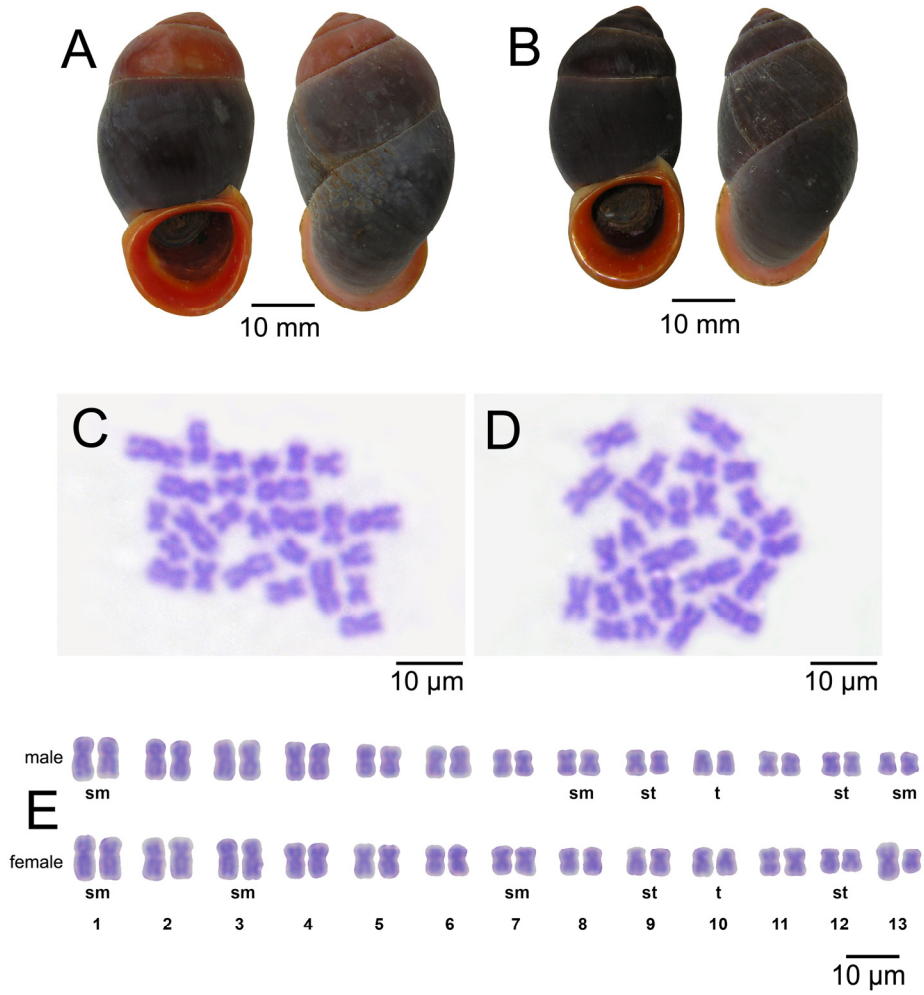

FIGURE 1. A, B. Shell morphology of *Pollicaria mouhoti*, (A) the typical form with the larger orange spired shelled form, from Tam Wangdang, Phitsanulok (CUMZ 1575) and (B) the smaller dark (monochrome blackish) shelled form,from Tam Pha Bing, Loei (CUMZ 1548). C, D. Mitotic chromosome spreads of the dark form of *Pollicaria mouhoti*, (C) male and (D) female specimens from Tam Pha Poo, Loei (CUMZ). (E) Karyotypes of male and female of the dark form of *Pollicaria mouhoti*; metacentric (un-labeled), submetacentric (sm), subtelocentric (st) and telocentric (t) forms.

Five species of *Pollicaria* have been studied from a cytogenetics viewpoint, of which of them have a haploid number of 13 chromosomes (Kongim et al., 2009), which is the basic figure for the family. Karyological based comparisons within populations can provide useful information for aiding taxonomic clarification (Kongim et al., 2006, 2009), such as the recent karyological studies of four *Pollicaria* spp. which indicated that the two forms of '*P. gravida* (Benson, 1856)' that have distinct karyotypic patterns should indeed be recognized as distinct taxa (Kongim et al., 2009). Similar argumentation has been confirmed in studies on other cyclophoroideans (Kongim et al., 2006).

In this study, the karyotypic pattern of the dark-shelled form of *P. mouhoti* is evaluated and compared with the previously reported typical form (Kongim et al., 2009). In addition the shell morphometric h/d ratio and whorl number were also evaluated.

TABLE 1. Localities, diploid number (*2n*), fundamental number (FN) and karyotype formula of the dark (sites 1 – 7) and typical (sites 8 – 13) forms of *Pollicaria mouhoti* (Pfeiffer, 1862) used in this study.

Locality	Number of Males, Females	(*2n*, FN)	Karyotype	Examined specimens
1. Phu Phalom, Loei	10, 10	26, 50	7m+3sm+2st+1t	CUMZ 1565
2. Tam Phasing, Loei	10, 10	26, 50	7m+3sm+2st+1t	CUMZ 1543
3. Phu Noi, Loei	10, 10	26, 50	7m+3sm+2st+1t	CUMZ 1575
4. Tam Pha Bing, Loei	10, 10	26, 50	7m+3sm+2st+1t	CUMZ 1548
5. Phu Phasamyod, Loei	7, 9	26, 50	7m+3sm+2st+1t	ZMMSU 0011
6. Khao Wangpha, Nongbualamphu	10, 10	26, 50	7m+3sm+2st+1t	CUMZ 1564
7. Tam Suwannakuha, Nongbualamphu	7, 7	26, 50	7m+3sm+2st+1t	ZMMSU 0007
8. Wat Pamamuang, Nernmaprang, Phitsanulok	10, 10	26, 50	6m+4sm+2st+1t	CUMZ 1541
9. Tam Wangdang, Nernmaprang, Phitsanulok	10, 10	26, 50	6m+4sm+2st+1t	CUMZ 1575
10. Namnow National Park, Phetchabun	5, 5	26, 50	6m+4sm+2st+1t	CUMZ 1574
11. Phu Phaman, Khon Kaen	6, 7	26, 50	6m+4sm+2st+1t	ZMMSU 0012
12. Phu Phachit, Chiayaphum	10, 10	26, 50	6m+4sm+2st+1t	ZMMSU 0013
13. Phu Kiew, Nongbuadang, Chiayaphum	10, 10	26, 50	6m+4sm+2st+1t	ZMMSU 0003

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

MATERIALS AND METHODS

The locality and number of snails karyotyped is given in Table 1. Species identifications were made on the basis of previous revisions (Kobelt, 1902; Pfeiffer, 1862), and compared with type specimens in the Natural History Museum, London (NHM).

For the chromosomes study, the method for collecting the metaphase cells, chromosome preparation and staining were as previously reported (Kongim et al., 2006, 2009). The animals were injected with 0.02% (w/v) colchicine 4 - 5 h before dissection. Gonad fractions were minced and subjected to hypotonic treatment for 15 - 20 min in 0.07% KCl solution. Material was fixed in a mixture of methanol and glacial acetic acid in a 3:1 (v/v) ratio. A cellular suspension was prepared by maceration of the material in a 3:2 (v/v) mixture of glacial acetic acid and distilled water, after which it was dropped onto dry slides and heated. The dried specimens were then stained for 10 min in 4% (w/v) Giemsa.

The mitotic karyotype images are arranged and numbered for chromosome pairs in order of decreasing mean relative length, with the nomenclature of morphological chromosome types following that of Levan et al. (1964). The diploid number (*2n*), fundamental number (FN) and karyotype formula of the dark and typical forms of *P. mouhoti* are presented in Table 1.

RESULTS

The mitotic karyotype of 13 populations of the dark form of *P. mouhoti* all exhibited the same number of haploid and diploid chromosomes, of $n = 13$, $2n = 26$, and the fundamental chromosome number (FN) occurred as 50 in all populations (Fig. 1C-E). Interestingly, the two shell forms of *P. mouhoti* from Thailand (Table 1 and Kongim et al. 2009 for further comparison) do not share the exact same karyotype, but differed by one in the number of metacentric and submetacentric chromosomes. The karyotype of the dark form of *P. mouhoti* from seven localities (Table 1, localities 1 - 7) in the northern area exhibited a (7m+3sm+2st+1t) karyotype with six large and seven median chromosomes, while the typical form specimens from six localities (Table 1, localities 8 - 13) from the southern area exhibited the karyotype formula of (6m+4sm+2st+1t) with nine large, three medium and one small chromosome. Similarly, a difference in the chromosome relative length (RL) and centromeric index (CI) were observed between the dark (RL = 0.05 - 0.10; CI = 6.84 - 50.00) and the typical (RL = 0.04 - 0.09; CI = 12.36 - 49.20) forms (Tables 2, 3).

Comparison of the karyotypes in females revealed distinct size heteromorphism of the metacentric and submetacentric chromosome pairs akin to ZW chromosomes (chromosome pair 13 in Fig. 1E). In contrast, all examined males have homologous pairs. Taken together, this is consistent with the presence of a ZZ–ZW type of sex chromosomes (ZZ in male, ZW in female), but this awaits confirmation.

DISCUSSION

The diploid chromosome number (*2n*=26) seen in the two forms of *Pollicaria mouhoti* analyzed here agree with that reported by Kongim et al. (2009) and in the related genus *Pupina* reported by Burch (1967). The other cyclophoroidean genera, *Cyclophorus*, *Theobaldius* and *Diplommatina*, also show a conserved chromosome number (Kongim et al., 2009; Kasinathan and Natarajan, 1975; Choundhury and Pandit, 1997).

TABLE 2. Chromosome measurement of the short arm (P), long arm (Q), total (TL), arm ratio (AR), relative length (RL) and centromeric index (CI) of ten cells used to determine the chromosome morphology of the dark morophological form of *P. mouhoti* (Pfeiffer, 1862) found in the northern part of Thailand.

Chromosome pair	P ± SD	Q ± SD	TL ± SD	AR ± SD	RL	CI	Chromosome morphology	Chromosome size
1	3.8 ± 0.26	6.5 ± 0.03	10.3 ± 0.35	1.72 ± 0.14	0.1034	36.8293	sm	L
2	4.5 ± 0.00	5.5 ± 0.02	10.0 ± 0.01	1.22 ± 0.00	0.1010	44.9800	m	L
3	4.8 ± 0.01	4.8 ± 0.02	9.6 ± 0.03	1.00 ± 0.00	0.0970	49.9740	m	L
4	4.6 ± 0.00	4.6 ± 0.01	9.1 ± 0.01	1.00 ± 0.00	0.0919	49.9863	m	L
5	4.5 ± 0.05	4.5 ± 0.05	9.0 ± 0.08	1.00 ± 0.00	0.0904	50.0000	m	L
6	4.4 ± 0.06	4.4 ± 0.06	8.7 ± 0.00	1.00 ± 0.00	0.0877	50.0000	m	L
7	3.8 ± 0.06	3.8 ± 0.05	7.6 ± 0.04	1.02 ± 0.01	0.0764	49.5050	m	M
8	1.8 ± 0.06	4.6 ± 0.06	6.3 ± 0.00	2.60 ± 0.00	0.0635	27.7778	st	M
9	2.1 ± 0.06	4.0 ± 0.05	6.0 ± 0.04	1.94 ± 0.02	0.0608	34.0249	sm	M
10	0.4 ± 0.12	5.5 ± 0.06	5.9 ± 0.07	13.63 ± 0.18	0.0590	6.8376	t	M
11	2.8 ± 0.06	3.0 ± 0.08	5.8 ± 0.07	1.09 ± 0.03	0.0580	47.8261	m	M
12	1.0 ± 0.06	4.8 ± 0.06	5.7 ± 0.00	5.00 ± 0.00	0.0575	16.6667	st	M
13	2.0 ± 0.06	3.4 ± 0.06	5.3 ± 0.00	1.72 ± 0.00	0.0535	36.7925	sm	M

Abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric; L, large; M, medium and S, small chromosomes.

TABLE 3. Chromosome measurement of the short arm (P), long arm (Q), total (TL), arm ratio (AR), relative length (RL) and centromeric index (CI) of ten cell used to determine the chromosome morphology of the typical morphological form of *P. mouhoti* (Pfeiffer, 1862) found in southern and central Thailand.

Chromosome pair	P ± SD	Q ± SD	TL ± SD	AR ± SD	RL	CI	Chromosome morphology	Chromosome size
1	4.3 ± 0.07	7.5 ± 0.07	11.7 ± 0.00	1.75 ± 0.01	0.0938	36.3539	sm	L
2	4.3 ± 0.06	7.5 ± 0.06	11.7 ± 0.00	1.75 ± 0.00	0.0936	36.3248	sm	L
3	4.2 ± 0.03	7.4 ± 0.04	11.6 ± 0.04	1.75 ± 0.01	0.0930	36.3441	sm	L
4	5.7 ± 0.05	5.9 ± 0.03	11.5 ± 0.02	1.04 ± 0.00	0.0923	49.0791	m	L
5	5.5 ± 0.06	5.6 ± 0.03	11.1 ± 0.00	1.03 ± 0.00	0.0886	49.2099	m	L
6	5.1 ± 0.06	5.8 ± 0.03	10.8 ± 0.00	1.14 ± 0.00	0.0866	46.6513	m	L
7	5.0 ± 0.03	5.6 ± 0.03	10.6 ± 0.00	1.13 ± 0.00	0.0848	46.9340	m	L
8	4.2 ± 0.05	5.5 ± 0.03	9.6 ± 0.02	1.32 ± 0.01	0.0771	43.1907	m	L
9	3.4 ± 0.03	5.8 ± 0.03	9.2 ± 0.00	1.71 ± 0.00	0.0732	36.8852	sm	L
10	1.9 ± 0.03	5.8 ± 0.03	7.7 ± 0.00	3.08 ± 0.00	0.0612	24.5098	st	M
11	1.8 ± 0.03	5.7 ± 0.03	7.6 ± 0.00	3.14 ± 0.00	0.0604	24.1722	st	M
12	0.8 ± 0.04	5.7 ± 0.03	6.5 ± 0.04	7.09 ± 0.31	0.0518	12.3552	t	M
13	2.4 ± 0.03	3.1 ± 0.09	5.5 ± 0.00	1.29 ± 0.00	0.0436	43.5780	m	S

Abbreviations: m, metacentric; sm, submetacentric; st, subtelocentric; t, telocentric; L, large; M, medium and S, small chromosomes.
chromosomes.

The karyotype of *P. mouhoti* obtained in this study suggests a diverged characteristic of karyotype formation for the dark form of *P. mouhoti*, compared to the typical form, that also matches their current geographic location. That is the dark form of *P. mouhoti* is the only recorded species in the northern part of Thailand, while the typical form have been recorded from the southern part of Thailand. Thus, *P. mouhoti* shows a karyotype diversification from metacentric to submetcentric, assuming the primitive karyotype has the lowest asymmetry and the derived karyotype the higher asymmetry (Diupotex-Chong et al, 2004). Under this scenario, the karyotype of the typical form shows the highest asymmetric chromosome pattern (6m+4sm+2st+1t) and is the derived character in the genus *Pollicaria*, whilst the dark form exhibits the lower asymmetric karyotype (7m+3sm+2st+1t).

Regardless, the karyotype differentiation between the two forms of *Pollicaria* is suggestive of them being different taxa. The karyotype descriptions of the other species of the Prosobranchia could be elucidated and any (fixed) chromosome changes used as a cytotaxonomic marker in this taxa. However, note that the differences in the centromeric indexes between some pairs of species are insufficient to reliably discriminate between them as different species, such as the case of the two allospecies of the genus *Planorbarius* which differ significantly at the centromeric indexes of the 12^{th} ($P = 0.0001$) and the 15^{th} ($P = 0.02$) pairs of chromosomes (Garbar and Garbar, 2007).

Karyotypic analysis can provide important evidence in differentiating taxa and lineages, and several of its parameters (primarily the centromeric indexes) may be useful for future taxonomic and cytogenetic studies of this snail group.

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