BIOCELL
2004, 28(3): 279-285

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Karyotype description of *Pomacea patula catemacensis* (Caenogastropoda, Ampullariidae), with an assessment of the taxonomic status of *Pomacea patula*

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Key words: apple snails, chromosomes, taxonomy, phylogeny, Mexico.

ABSTRACT: Mitotic chromosomes of the freshwater snail *Pomacea patula catemacensis* (Baker 1922) were analyzed on gill tissue of specimens from the type locality (Lake Catemaco, Mexico). The diploid number of chromosomes is 2n = 26, including nine metacentric and four submetacentric pairs; therefore, the fundamental number is FN = 52. No sex chromosomes could be identified. The same chromosome number and morphology were already reported for *P. flagellata,* i.e., the other species of the genus living in Mexico. The basic haploid number for family Ampullariidae was reported to be n = 14 in the literature; so, its reduction to n = 13 is probably an apomorphy of the Mexican *Pomacea* snails. *Lanistes bolteni,* from Egypt, also shows n = 13, but its karyotype is much more asymmetrical, and seems to have evolved independently from *P. flagellata* and *P. patula catemacensis*. The nominotypical subspecies, *P. patula patula* (Reeve 1856), is a poorly known taxon, whose original locality is unknown. A taxonomical account is presented here, and a Mexican origin postulated as the most parsimonious hypothesis.

Introduction

Apple snails (family Ampullariidae) constitute a well-defined monophyletic group of freshwater mollusks, its members sharing more than 20 synapomorphies (Berthold, 1991; Bieler, 1993) within the Caenogastropoda Architaenioglossa (in the sense of Ponder and Lindberg, 1997). Most species of this family live in tropical and subtropical ecosystems of the Southern Hemisphere across Africa, Asia and America. *Pomacea* Perry 1810 is the most representative American genus, with 117 not yet synonymized nominal species, though the real number of species is probably near 50 (Berthold, 1989; Cowie and Thiengo, 2003).

Just a few species of *Pomacea* live to the north of 15ºN latitude and only two species are at present recognized in Mexico, namely, the widespread *Pomacea flagellata* (Say 1827) and the strictly endemic *P. patula catemacensis* (Baker 1922). The latter (locally named

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Received on October 27, 2003. Accepted on July 28, 2004.

"tegogolo") shows considerable variation among individuals both in size and color of the shell; these organisms are suitable models for genetic research and breeding experiments, due to their short life cycle, high hatching rate and number of eggs, and easy rearing (Martínez, 1989). They are also an important food resource in the area of Lake Catemaco (Veracruz-Llave State, Mexico), which is its type and only known locality (Naranjo-García and García-Cubas, 1986).

Only a few species of *Pomacea* have been studied from a cytogenetic viewpoint, and most authors have reported a haploid number of 14 chromosomes (Brand *et al.,* 1990; Kawano *et al.,* 1990; Mercado-Laczkó and Lopretto, 1998), which is the basic figure for the family according to Choudhury and Pandit (1997). Mexican species of *Pomacea* seem to make an exception however, since *P. flagellata* has a haploid number of 13 (Diupotex-Chong, 1994), and the same figure has been reported also for *P. patula catemacensis* (Yam, 1986; Diupotex-Chong *et al.,* 1997). Sharing this character may be meaningful within the context of the current hypotheses of relationships among the American Ampullariidae.

The aim of this study is to describe the karyotype of *Pomacea patula catemacensis,* and to analyze its significance in the context of the phylogeny and taxonomy of the genus. A preliminary analysis of the taxonomic status of *Pomacea patula* (Reeve 1856), is also included.

Materials and Methods

Sixty live specimens of *Pomacea patula catemacensis* were collected from Lake Catemaco (18º 24' N – 95º 04' W), and reared in the laboratory until used for cytogenetic analysis.

Chromosomes from gill tissue in mitotic metaphase were mounted following the techniques described by Kligerman and Bloom (1977). The animals were injected with 0.5 ml of a 0.075 M KCl hypotonic solution, and were injected again 2 hours later with 0.5 ml of colchicine (0.02%). Two hours after the second injection, gills were excised and chopped.

Tissue pieces were placed into bi-distilled water for 20-30 min and fixed in at least two changes of fixative 3:1 methanol-acetic acid (freshly mixed) for 24 hours at 4ºC. The tissues were then minced gently and placed in 60% acetic acid for 30 min to prepare a cell suspension, which was placed onto a clean slide with a capillary tube (75 mm x 1.2 mm), and heated at 60ºC.

Staining was performed with 5% Giemsa in 0.1 M phosphate buffer, pH 6.8, and mounted in Canada balsam. Observations were made and micrographs were taken with a Carl Zeiss microscope. Fresh slides of gonadal tissue showing diakinesis phases were also analyzed under a phase contrast microscope to confirm the haploid number.

The relative length of each chromosome was expressed as a percentage of the absolute length of each chromosome pair out of the total length of the chromosome complement. The centromeric index was calculated as a percentage of the length of the short arm out of the total length of the chromosome. The arm ratio was calculated as the quotient long arm length/ short arm length (Q/P), and the chromosomes classified according to the terminology of Levan *et al.* (1964), i.e., chromosomes are named metacentric when they have a mean arm ratio of up to 1.7; submetacentric up to 3.0; subtelocentric up to 7.0; and telocentric over 7.0. Karyotype asymmetry was described following Stebbins (1950, 1971), and further assessed using the asymmetry indices A_1 and A_2 defined by Romero-Zarco (1986). A_1 estimates the intrachromosomal asymmetry as $A_1 = 1 - \frac{\sum_{i=1}^{n} \frac{\overline{P}_i}{\overline{Q}_i}}{n}$ where n is the number of homologous chromosome pairs; $\overline{P}_i$ is the average length for short arms in every chromosome pair, and $\overline{Q}_i$ is the average length for long arms in every chromosome pair. A_2 depicts the interchromosomal asymmetry as the ratio of the standard deviation to the mean length of the chromosomes. Karyotype asymmetry was compared

FIGURE 1. Metaphase chromosome spread of *Pomacea patula catemacensis* (Baker 1922). Scale bar = 5 μm.

with data published by previous authors on species of Ampullariidae.

The presence of sex chromosomes was searched by looking for morphologically different heterosomes and/or heteropicnotic chromosome regions (Óstergren, 1950).

Results

Figure 1 shows a mitotic metaphase plate of gill tissue of *Pomacea patula catemacensis*, with 13 pairs of chromosomes, nine of which are metacentric and four submetacentric (Table 1). Diakinesis phase observed in squashes of fresh gonadal tissue confirmed the haploid number n = 13. Figure 2 shows the karyotype arranged by decreasing chromosome size; it reveals balanced size and form values, for both the P and Q arms. According to their form and architecture, the chromosomes evi-

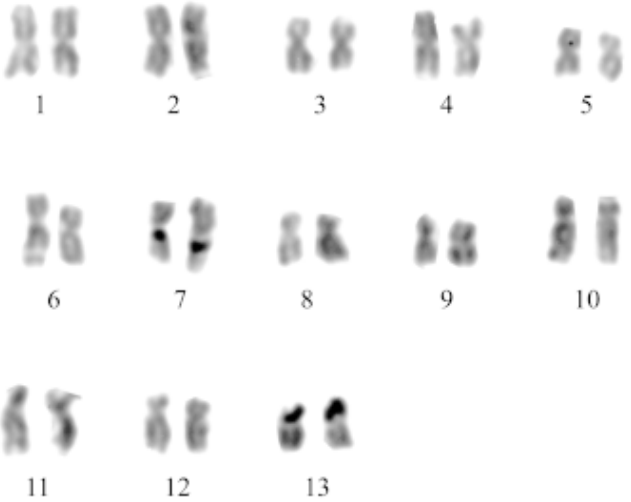

FIGURE 2. Representative karyotype of *Pomacea patula catemacensis* (Baker 1922).

TABLE 1.

Relative chromosome lengths and arm ratios in *Pomacea patula catemacensis,* from n = 15 representative metaphases. $\overline{P}$ = mean length of the short arm (μm); $\overline{Q}$ = mean length of the long arm (μm); SD = standard deviation; TL = absolute total length ($\overline{P} + \overline{Q}$); CI = centromeric index ($100\,\overline{P}/TL$); $\overline{RL}$ = mean relative length: $\frac{1}{n}\sum(100TL/\sum TL)$; $\overline{AR}$ = mean arm ratio: $\frac{1}{n}\sum(Q/P)$; m = metacentric chromosomes; sm = submetacentric chromosomes.

Chromosome pair	$\overline{P} \pm$ SD	$\overline{Q} \pm$ SD	TL	CI	$\overline{RL} \pm$ SD	$\overline{AR} \pm$ SD	Chromosome morphology
1	3.1 ± 0.72	3.7 ± 0.30	6.8	44.66	10.64 ± 1.9	1.24 ± 0.10	m
2	2.5 ± 0.44	3.5 ± 0.24	6.0	41.21	9.42 ± 2.0	1.42 ± 0.15	m
3	2.4 ± 0.42	3.2 ± 0.33	5.6	43.17	8.78 ± 2.5	1.32 ± 0.17	m
4	2.2 ± 0.40	3.2 ± 0.33	5.4	40.71	8.49 ± 3.4	1.46 ± 0.13	m
5	2.2 ± 0.17	2.9 ± 0.22	5.1	42.1	8.02 ± 4.1	1.32 ± 0.24	m
6	2.1 ± 0.11	2.8 ± 0.32	4.9	41.4	7.44 ± 4.1	1.42 ± 0.23	m
7	2.1 ± 0.14	2.5 ± 0.30	4.6	44.12	6.98 ± 2.4	1.27 ± 0.16	m
8	1.9 ± 0.24	2.2 ± 0.25	4.1	45.5	6.49 ± 3.2	1.16 ± 0.12	m
9	1.8 ± 0.11	2.1 ± 0.35	3.9	47.36	5.97 ± 4.5	1.11 ± 0.11	m
10	1.8 ± 0.11	3.4 ± 0.21	5.2	34.24	8.12 ± 5.3	1.92 ± 0.22	sm
11	1.5 ± 0.12	3.1 ± 0.33	4.6	32.8	7.01 ± 4.2	1.87 ± 0.13	sm
12	1.6 ± 0.13	2.9 ± 0.34	4.5	34.89	7.11 ± 2.2	1.73 ± 0.24	sm
13	1.5 ± 0.13	2.5 ± 0.29	4.0	37.53	6.28 ± 3.1	2.05 ± 0.29	sm

denced a clear homologous setup by their relative length and centromeric index values. The fundamental number was NF = 52. Figure 3 depicts the ideogram constructed from the relative length and centromeric index values.

Karyotype asymmetry corresponds to the category A2 of Stebbins (1971), since the ratio of the largest to the smallest chromosome in the karyotype was 1.74, and only one out of the 13 chromosomes (8%) showed an arm proportion <2:1. The intrachromosomal asymmetry index (A_1) was 0.29, while the interchromosomal index (A_2) was 0.17.

Sex chromosomes were not detected in our material, by observation neither of heterosomes, nor of heteropycnotic elements.

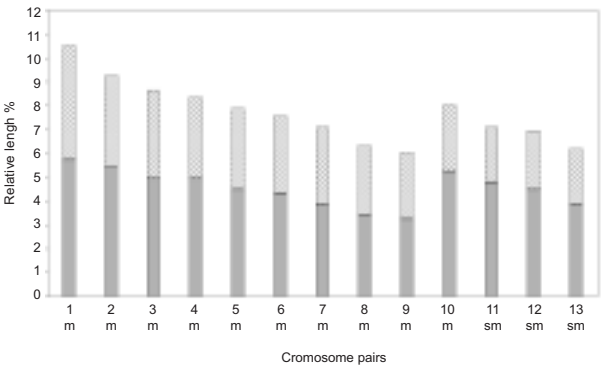

FIGURE 3. Ideogram of *Pomacea patula catemacensis* chromosomes.

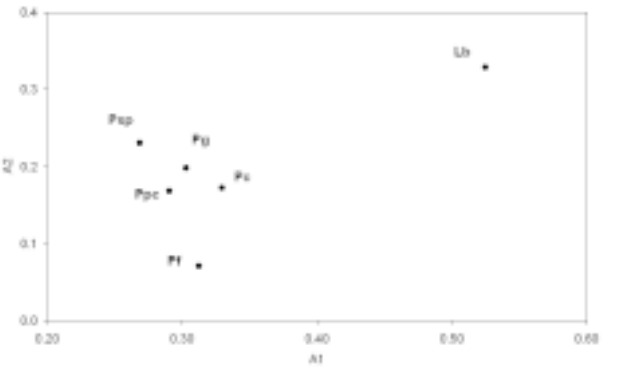

FIGURE 4. Karyotype asymmetry (as defined by Romero-Zarco, 1986) in six species of Ampullariidae. A_1, intrachromosomal asymmetry index; A_2, interchromosomal asymmetry index; Lb, *Lanistes bolteni,* Egypt (data after Yaseen *et al.*, 1991); Pc, *Pomacea canaliculata,* Argentina (after Mercado-Laczkó and Lopretto, 1998); Pf, *Pomacea flagellata,* Mexico (after Diupotex-Chong, 1994); Pg, *Pila globosa,* India (after Choudhury and Pandit, 1997); Ppc, *Pomacea patula catemacensis,* México (this study); Psp, *Pomacea* sp., Brazil (after Kawano *et al.*, 1990).

Discussion

Family Ampullariidae includes 13 genus-group named taxa; their phylogenetic relationships were analyzed by Berthold (1989, 1991) and Bieler (1993). Current hypotheses of ancestry advocate that all Neotropical genus-group taxa (namely, *Asolene* d'Orbigny 1837, *Effusa* Jousseaume 1889, *Felipponea* Dall 1919, *Marisa* Gray 1824, *Pomacea* Perry 1810, *Pomella* Gray 1847, and *Surinamia* Clench 1933) constitute a single monophyletic group, whose sister group is genus *Pila* Röding 1798, from Africa and Asia.

All the cytogenetically studied species of *Pila* have a haploid number of n = 14. This was the figure reported for *Pila ovata* (Oliver 1804) from Egypt (Lufty and Demian, 1965), and *Pila virens* (Lamarck 1819) from India (Ramamoorthy, 1967, cited by Choudhury and Pandit, 1997); no details on chromosome morphology were given in either case. A set of nine metacentric and five submetacentric chromosomes was described for *Pila globosa* (Swainson 1820) from India by Choudhury and Pandit (1997), who consider n = 14 to be the basic haploid number for the family. The same was reported for the Neotropical snail *Marisa cornuarietis* introduced into Egypt (Lufty and Demian, 1965), for *Pomacea* sp. from Southern Brazil (Kawano *et al.,* 1990), and for *Pomacea canaliculata* from both native realm in South America and from Japan, where it was introduced in the eighties (Brand *et al.*, 1990; Mercado-Laczkó and Lopretto, 1998). Instead, the two Mexican species of *Pomacea* have n = 13 (Yam, 1986; Diupotex-Chong, 1994; Diupotex-Chong *et al.*, 1997; this study), which may represent an apomorphic condition within the American clade.

Berthold (1991) concluded that *Marisa* is the sister group of *Pomacea sensu lato,* i.e., *Pomacea (Pomacea) + Pomacea (Effusa)*. On the other hand, Bieler's (1993) strict consensus tree shows *Marisa* and *Effusa* as a clade that shares a more ancient ancestor with *Pomacea s.s.* Regrettably, there is no information on the karyotype of most ampullariids, and so we can only speculate that the coincidence of n = 13 in the two

species of *Pomacea* living in Mexico may be a synapomorphy of the representatives living in the northern range of the genus. This hypothesis needs to be tested by studying the karyology of *P. paludosa* (Say 1829)*, P. cubensis* (Morelet 1849), and *P. poeyana* (Pilsbry 1927), i.e., the native species of *Pomacea s. s.* that lives further northwards.

Shell morphology is often influenced by environmental conditions, geographic barriers, age of the organisms, and other factors affecting adaptation and selection (Inaba, 1961; Burch, 1960, 1967). Other sources of information may be enlightening, although incompatibility of specific delimitations based on different kinds of data are frequent. For example, while trying to characterize the species of *Pomacea* introduced into Thailand, Keawjam and Upatham (1990) demonstrated that some organisms with different shell or anatomic features may produce similar genetic patterns, whereas individuals that have almost identical morphology differ genetically. Number and morphology of the chromosomes may provide specific, crucial evidence for solving some taxonomic problems (White, 1973).

Brand *et al*. (1990) studied the chromosomes of a *Pomacea* species introduced into Japan, where it became an agricultural pest. The specimens were identified as *Pomacea canaliculata* (without any description, taxonomically useful illustration, or specific collection locality). A diploid chromosome formula of 20 m + 6 sm + 2 st was described for females, and 19 m + 7 sm + 2 st for males. Based on this limited information, and in the absence of other judgment resources, we conclude that the Japanese specimens were probably closer to the Brazilian specimens studied by Kawano *et al*. (1990) than to the Buenos Aires *P. canaliculata*.

Probably more than one closely related species of *Pomacea* were introduced into Japan, including both *P. canaliculata* and *P. lineata* (Cowie, 2002). Whichever their taxonomic relationships, the presence or absence of a subtelocentric pair in the diploid complement may be an argument for recognition, provided the constancy of this character is confirmed by further studies.

Karyotypes with a higher proportion of metacentric chromosomes, as shown by *Pomacea patula catemacensis*, are probably primitive, and show relative chromosome stability (White, 1951, 1978).The intrachromosomal asymmetry index is almost constant in the [*Pila* + Neotropical apple snails] clade (A1= 0.27-0.31), i.e., five out of the six studied Ampullariid taxa (Fig. 4). The lowest value was calculated for an unidentified species from São Paulo (Brazil), probably *Pomacea lineata* (Spix 1827) whose chromosome formula is 9 m + 4 sm + 1 st (Kawano *et al.,* 1990), while the highest asymmetry within this genus corresponds to *Pomacea canaliculata* (Lamarck 1822) from Buenos Aires Province (Argentina), with 11 metacentric and 3 submetacentric chromosomes (Mercado- Laczkó and Lopretto, 1998).

The sister group of the clade formed by *Pila* plus the Neotropical forms is the African, hyperstrophic genus *Lanistes sensu lato* (i.e., *Lanistes* + *Plesiolanistes* + *Pseudoceratodes*). Only one species of this group, identified as *Lanistes bolteni* from Egypt, has been studied from a karyological viewpoint, with conflicting results. While Lufty and Demian (1965) reported n = 14, without details on chromosome morphology and measurements, Yaseen *et al.* (1991) reported n = 13, with a chromosome formula 8 m + 2 st + 3 t. The presence of two subtelocentric and three telocentric chromosomes in *Lanistes* reveals a much more specialized and asymmetrical condition, suggesting that the reduction of its chromosome number to 13 is a non-homologous event, independent from the reduction to n = 13 in the Mexican species.

Brand *et al.* (1990) described the existence of male heterosomes X-Y, one metacentric and one submetacentric, which determine sex in *Pomacea canaliculata* from Japan. Such a heterogamety was not detected by other authors, neither in *Pomacea,* nor in other ampullariid genera; no sex chromosomes were identified in any Mexican species of *Pomacea* either (Diupotex-Chong, 1994; this study). It is evident that sex determination in these snails still needs further research. Yusa and Suzuki (2003) mentioned that a polyfactorial system of sex determination may exist in *Pomacea,* although it is also possible that different species or populations have different mechanisms.

Preliminary assessment of Pomacea patula (Reeve 1856).

Identifying and delimiting ancient morphospecies is a difficult task, especially when a type locality has not been stated (Cazzaniga, 2002). *Pomacea patula* is a poorly known species, described solely on the basis of its shell outline; it was at first compared only to *Ampullaria neritoides* d'Orbigny 1835 on the grounds of having an oddly broad aperture. The latter species is now recognized as a synonym of *Pomella megastoma* (Sowerby 1825) (Jaeckel, 1927; Pilsbry, 1933; Hylton-Scott, 1958), and its superficial similitude with *P. patula* proved not to be of taxonomic bearing. The original publication of *P. patula* included no data on the origin

of the material, not even the continent where the species lived. The species was later omitted from most catalogues and taxonomic works (e.g., Sowerby, 1909; Alderson, 1925). Furthermore, the name was pre-occupied by *Ampullaria patula* Lamarck 1804 (now placed in genus *Globularia*, Naticidae), and so, *A. patula* Reeve 1856 is a junior primary homonym. However, no nomenclatural act is here included, waiting for a further study of the case.

To state that his new subspecies *catemacensis* fitted in *P. patula*, Baker (1922) compared it to a couple of shells collected in Mexico and labeled as *P. patula* in the Academy of Natural Sciences of Philadelphia collection. He acknowledged that these shells and *P. patula catemacensis* belong to the *Ampullaria ghiesbrechtii* group, i.e., the "*Pomacea flagellata* complex" (Pain, 1964), which is now considered a single, extremely variable species extending from Mexico and Central America to the Magdalena drainage area, in northern Colombia (Bequaert, 1957; Branson and McCoy, 1963; Rangel-Ruiz, 1988). Our cytogenetic results do not conflict with the relatedness of *catemacensis* and *flagellata,* which also has a haploid complement of 13 metacentric and submetacentric chromosomes (Diupotex-Chong, 1994).

Pomacea patula has been also mentioned as living in Brazil (a quotation from a personal communication by B. Walker, in Baker, 1922: 39). However, this conclusion was very weakly founded. Dr. Walker compared Baker's pencil-sketched figures to the Reeve's published figure, and referred that he also had four South American lots labeled "*patula*": two of them collected in "New Granada", i.e., Colombia plus Panama, a region where it is feasible to find *P. flagellata*- like apple snails; he was at the time unable to trace the third lot, collected from the Amazon, while the fourth lot, supposedly coming from Brazil, was "dealer's specimens, and know nothing of their history" (Walker in Baker, 1922: 39 footnote). Therefore, there was no well-documented evidence of the presence of *P. patula* in Brazil.

Besides, later reports on the ampullariids from northern South America and the Amazon region failed to include *P. patula* (e. g., Baker, 1930; Bequaert, 1925; Geijskes and Pain, 1957; Haas, 1949, 1951; Jaeckel, 1952; Jousseaume, 1889; Martens, 1873; Pain, 1950, 1952, 1956, 1957, 1960; Pilsbry, 1933), except for a single mention in a checklist of a mollusk collection from the Mato Grosso region (Lopes, 1957) with no description or other data supporting this identification. The presence of a representative of the *Pomacea flagellata* group in the Amazon basin would be fairly astounding, and so the identity of the Mato Grosso *P. patula* should be reassessed. The concurrence of *flagellata*-like shell features and our karyological data hint at a Mexican origin for *P. patula patula* (Reeve 1856) as the most parsimonious hypothesis.

Acknowledgments

We would like to thank M.S. Mario Alejandro Gómez Ponce for the collection of organisms, Dr. Manuel Uribe Alcocer for his analysis suggestions, and Lea Cazzaniga for revising and improving the English wording. NJC is a member of the Scientific Researchers Career of the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina.

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