

## Workshop: Biology of Ampullariidae

### Conchological variation in *Pomacea canaliculata* and other South American Ampullariidae (Caenogastropoda, Architaenioglossa)

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**Key words:** shell, operculum, periostracum, banding pattern, apple snail

*Pomacea canaliculata* (Lamarck, 1822) is a freshwater snail belonging to the family Ampullariidae, a taxon that includes Asian, African and American species collectively known as apple snails. *P. canaliculata* ranges from the Amazonas Basin (Brazil) to Tandilia and Ventania Mountain systems (Buenos Aires province, Argentina) and is the most widely studied snail in Argentina, being the object of different morphological, anatomical, ecological, embryological and taxonomical studies (Catalán *et al.*, 2002; Castro-Vazquez *et al.*, 2002; Estebenet and Martín, 2002; Cazzaniga, 2002; Heras and Pollero, 2002). International concern about *P. canaliculata* rose enormously when it became established as a serious rice pest in Asia (Cowie, 2002). Asian pest managers were very much troubled with the identification of the invading apple snails (Wada, 1997), a task seriously complicated by the multiple origin of invaders and their great morphological and ecophysiological

variability (Cowie, 2002; Estebenet and Martín, 2002, 2003; Martín and Estebenet, 2002). Although already recognized by d'Orbigny (1835-1846), the wide conchological variation of *P. canaliculata* has been seldom quantitatively studied and most studies on the subject were performed in a restricted area (Buenos Aires province) in the southern area of its natural distribution (Cazzaniga, 1990; Estebenet, 1998; Estebenet and Martín, 2003).

The shell of *P. canaliculata* has been described as globose to subglobose, with a low spire and an oval aperture; the color of the shell is brown-green, showing several dark spiral bands of variable width and transverse growth lines (Castellanos and Fernández, 1976). The operculum is corneous with concentric growth lines around an excentric nucleus. Most of these traits, and many others, show a great influence of ontogenetic, sexual, genetic and ecophenotypic components, which give place to an important intra- and interpopulation variation. The aims of our study are to describe and analyze the variation and the origin of *P. canaliculata* shell traits, and to compare them with the information available for other Neotropical Ampullariids, focusing mainly on quantitative or experimental studies.

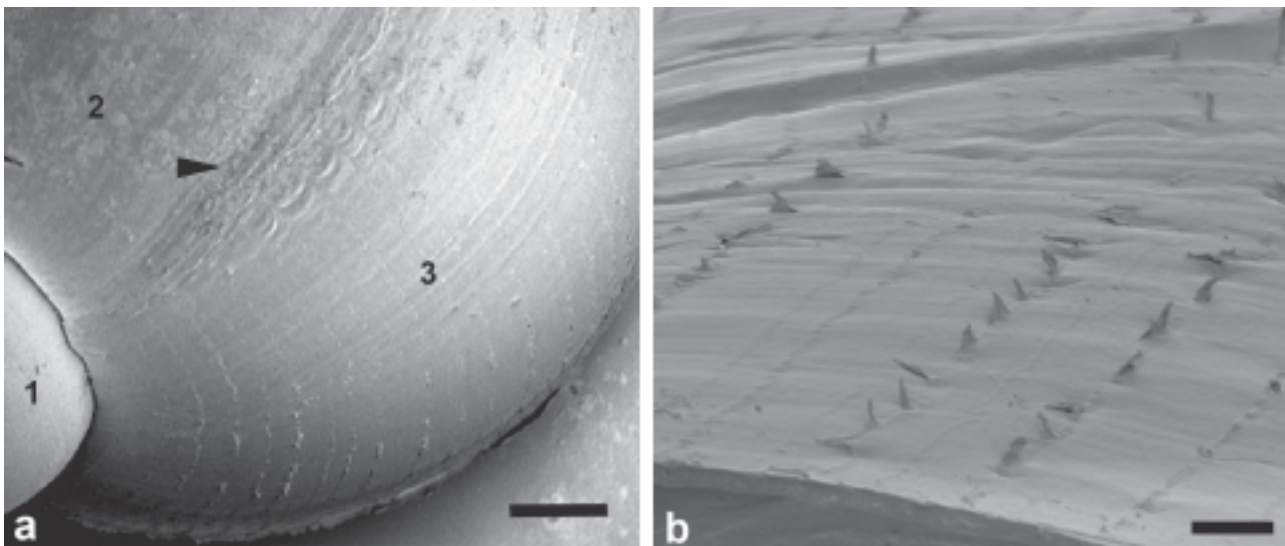
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### Shell periostracum

Periostracal hairs disposed in spiral series are common in *P. canaliculata* and have been recorded in other apple snail species (Berthold, 1991). In *P. canaliculata* we observed that the protoconch and the pre-hatching teleoconch are devoid of periostracal hairs and that spiral rows of thin triangular flakes appear early in the post-hatching teleoconch (Fig. 1). Periostracal hairs are also quite evident in newly deposited portions of the shell of post-hibernating adult field snails and apparently abraded in older portions. Extremely hirsute juvenile snails, probably *P. scalaris* (d'Orbigny, 1835) are occasionally observed (Cazzaniga, pers. comm.). Both embryonic and juvenile periostracal hairs are well developed in the sister family Viviparidae, showing important interspecies and intergenus differences (Jokinen, 1984). Periostracal hairs, though lost in later stages, can be used in identification of newborn or juvenile viviparids (Ribi and Oertli, 2000), but the information on the fine morphology of these structures in Ampullariidae is insufficient yet to know if it could be used for this purpose. Berthold (1991) suggested that periostracal hairs could play a role in the homogenization of intracapsular fluid in Ampullariids, though this is not the case at least in *P. canaliculata*, since the shell of intracapsular stages is notably smooth.

### Shell chirality

Sinistral coiling of the shell in *P. canaliculata* is extremely rare: only one female has been reported from an artificial pond in La Plata city, that showed also an inverted body organization (Cazzaniga and Estebenet, 1990). After fifteen years of intensive field and laboratory work with thousands of specimens of this species only one new sinistral specimen has been found: an adult male with the same inverted body organization, retrieved in La Corina stream, a small watercourse in Buenos Aires province. Both specimens were unable to copulate even when grouped with normal dextral snails of the opposite sex, probably due to the inability of the males to find the gonopore of the female partner when located in the other side of the body. However, copulation between individuals with opposite chirality is possible among those pulmonate snails in which the individual playing the male role mounts the shell of the one playing the female role (Asami *et al.*, 1998). If inverted body organization has a genetic basis as in other snails, then this would preclude the transmission or conservation of "inverting" alleles and could explain the very low frequencies of sinistral shells. Sinistral coiling is also exceptional in the family Ampullariidae as a whole (Cazzaniga and Estebenet, 1990).



**FIGURE 1.** Scanning electron micrographs of critical-point dried shells of newly hatched *Pomacea canaliculata* (less than three days old): a) dorsal view (1: protoconch, 2: pre-hatching teleoconch, 3: post-hatching teleoconch, arrowhead: hatching mark; scale bar: 200  $\mu\text{m}$ ); b) detail of periostracal hairs near the apertural lip (scale bar: 40  $\mu\text{m}$ ).

## Shell Color

The banding pattern is highly variable among individuals from the same population of *P. canaliculata*. This variation involves the color, the intensity, the number (up to 30 bands) and the width of bands. In some populations from Southern Buenos Aires province, unbanded individuals are not infrequent in any given large sample of snails. However, apparently unbanded individuals that appeared in our laboratory stocks from one of these populations showed a very weak banding pattern (both in intensity and number of bands), visible only after a close examination of the empty shells under proper illumination.

The presence of bands is under the control of a single locus gene in the giant ramshorn snail *Marisa cornuarietis* (Linné, 1758), with the unbanded condition (“golden”) recessive; the inheritance of banding is independent of that of body color, being the “golden” snails indistinguishable from the wild phenotypes in all other respects (Dillon, 2003), although this variant has not been recorded in field populations. The bands are absent also in albino *P. canaliculata* snails (“yellow”) that lack dark pigments in the skin, the eyes and the shell, a recessive condition with simple Mendelian inheritance (Yusa, 2004). Unbanded and albino strains of *Pomacea* spp. generated and maintained in the aquarium trade are common in Europe, North America and Asia (Perera and Walls, 1996; Raut and Aditya, 1999) and probably have been the source of “golden” apple snail variants that now thrive in the wild in some of these regions (Dillon, 2003).

Bands darker than the background are a frequent feature in ampullariid shells. According to some authors the pigment of the bands is deposited in the periostracum (Castellanos and Fernández, 1976; Cazzaniga and Estebenet, 1990) although this is not the common rule among gastropods, in which the pigments are produced by specialized cells in the mantle margin and deposited in the outer calcareous layer or ostracum. In *P. canaliculata* at least, the bands are included within the calcareous shell matrix, as can be proved by acid dissolution of the shell, which leaves only a homogeneously brownish-colored periostracum (pers. obs.); on the other hand the chemical digestion of the periostracum only fades the general shell coloration, leaving the bands unaltered.

The banding pattern variation shows ontogenetic and ecophenotypic components: the color intensity of the bands in *P. canaliculata* increases during posthatching development, and the shells of hatchlings have no bands.

Fast growing laboratory snails develop thinner (Estebenet and Martín, 2003) and at the same time weaker-banded shells than their source field populations. Moreover, in many field snails the banding pattern suddenly appears after a distinct shell growth mark. Perhaps the intensity of the bands is directly related to shell thickness.

## Shell shape

Ontogenetic growth patterns of the shell of *P. canaliculata* have been studied only for a population from Paseo del Bosque pond, La Plata city (Estebenet, 1998). The shell shows a gentle though definitely allometric growth in many dimensions: shell width, aperture width and spire length grow faster than total length while aperture length grows slower; the overall shape of the shell becomes more globose and the aperture wider during ontogeny. These patterns are valid for snails larger than 9.0 mm of shell length, which are oblong. However, newborn are almost isodiametric (Hylton-Scott, 1958; Estebenet and Cazzaniga, 1993) so the early post-hatching growth patterns must show the opposite tendency.

A sexual component of intrapopulation morphological variation of *P. canaliculata* has also been described although the sexual dimorphism involves only the aperture shape; as in *Pomacea urceus* (Müller, 1774) (Burky, 1974), the main proportions of the shell are not different. The specific growth rate of aperture width increases in males during maturity, so the male aperture growth pattern diverges from that of females, which is almost continuous with that of juveniles (Estebenet, 1998), resulting in a wider male aperture (Cazzaniga, 1990). A similar but somewhat more pronounced sexual dimorphism was described in the planispiral shell of *M. cornuarietis* (Demian and Ibrahim, 1972). The development of the penis sheath complex, located in the mantle margin, may result in this sexual differentiation of the aperture growth pattern (Demian and Ibrahim, 1972; Cazzaniga, 1990; Estebenet, 1998), and hence would be a feature of other ampullariid species. Probably, the scarcity of reports of sexual shape dimorphism is only the result of the few specific studies aimed on the subject and the subtleness of the differences, rarely perceptible to an inexperienced eye (Perera and Walls, 1996). The degree of sexual shape dimorphism in *P. canaliculata* shows a wide interpopulation variation (unpub. data).

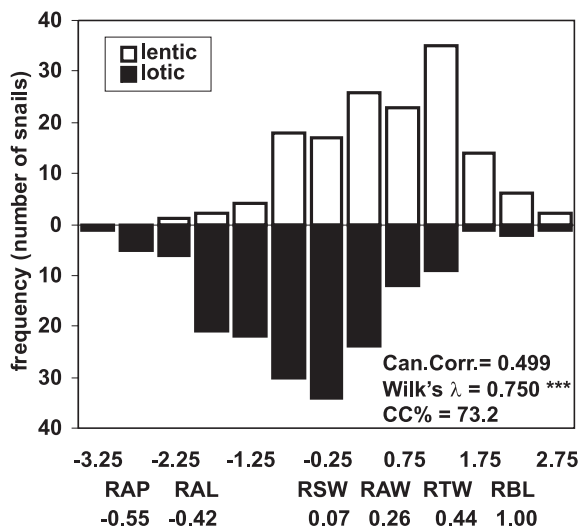
The morphological variation shows a strong inter-

population component in *P. canaliculata* (Cazzaniga, 1987, 2002), that is the result of environmental factors and genetic differentiation (Estebenet and Martín, 2003). Snails reared from egg masses collected at three different populations located in the same basin in Southern Buenos Aires province showed significant shape differences when reared under homogeneous conditions in the laboratory, indicating a genetic basis for this shell shape variation. On the other hand, adult snails collected in these same populations showed different shell shapes than their laboratory counterparts, suggesting environmental influences on shell growth patterns. The field snails seem to show a greater interpopulation morphological variation than their descendants reared in the laboratory, probably due to the interaction of ecophenotypic, genetic and also allometric components, since great size differences among populations exist.

The genetically based differences in shell shape among lentic and lotic populations do not seem to be the result of local adaptation to different flow regimes but a collateral outcome of adaptive differences in some life history traits (Martín and Estebenet, 2002). For example, the higher oviposition rate and clutch sizes in

one of the populations imply longer egg-laying periods out of water, during which the shape of mantle margin is altered and consequently the deposition of new shell material along the reproductive life.

Although the above mentioned study proved the existence of an ecophenotypic component on shell shape, the identity of the environmental factors responsible of the variation among populations remained elusive. An ongoing study on many populations of *P. canaliculata* from a wider spectrum of waterbodies belonging to different basins and distributed over all Southern Buenos Aires provided some information on the environmental factors that could affect shell shape. Discriminant analysis based on six lineal dimensions of the shell adjusted by size were performed to detect significant shell shape differences between contrasting types of waterbodies. Shell shape of both males and females from lotic and lentic habitats differed significantly (Fig. 2). There were also significant differences in shell shape of both sexes between lakes and reservoirs with hard bottoms located on hilly terrains and shallow lakes with sandy bottoms. Among the lotic waterbodies, shell shape was different between those with sand-muddy bottoms and those with limestone bottoms for both sexes. Each of the habitat types used in the precedent analysis contained waterbodies from different drainage basins, suggesting that the environmentally based variation in shell shape overrides the genetically based variation that would result from isolation or genetic drift. This suggests that water flow and consistency of substrates affect the growth patterns of the shell, resulting in widely overlapping ecophenotypic morphs appearing as an almost continuous variation.



**FIGURE 2.** Frequency histogram of scores and standardized coefficients of the canonical function of the discriminant analysis between females from lentic and lotic waterbodies in Southern Buenos Aires province, based on the following shell ratios: RAP = AP/TL, RAL = AL/TL, RS = SW/TL, RAW = AW/TL, RBL = AP/TL (TL: total length, AP: apertural projection, AL: apertural length, SW: spire width, AW: apertural width, TW: total width, BL: body whorl length). (Can. Corr.: canonical correlation coefficient; CC%: percentage of correctly classified cases; \*\*\*:  $p < 0.001$ ).

### Shell size and weight

Sexual dimorphism in shell length has been recorded in populations inhabiting a small stream tributary of the La Plata river (Martín, 1984) and an artificial pond in the same area (La Plata city, Estebenet and Cazzaniga, 1998); in both cases the females showed higher mean shell lengths than males. However, mean shell lengths were not significantly different between sexes in one lentic and two lotic populations from Southwestern Buenos Aires province, even though females grew larger than males in the laboratory (Estebenet and Martín, 2003). Similar dimorphic growth patterns have been reported in all the experimental cohorts hitherto studied (Estebenet and Cazzaniga, 1998; Tanaka *et al.*, 1999; Estoy *et al.*, 2002) but the sexual dimorphism can

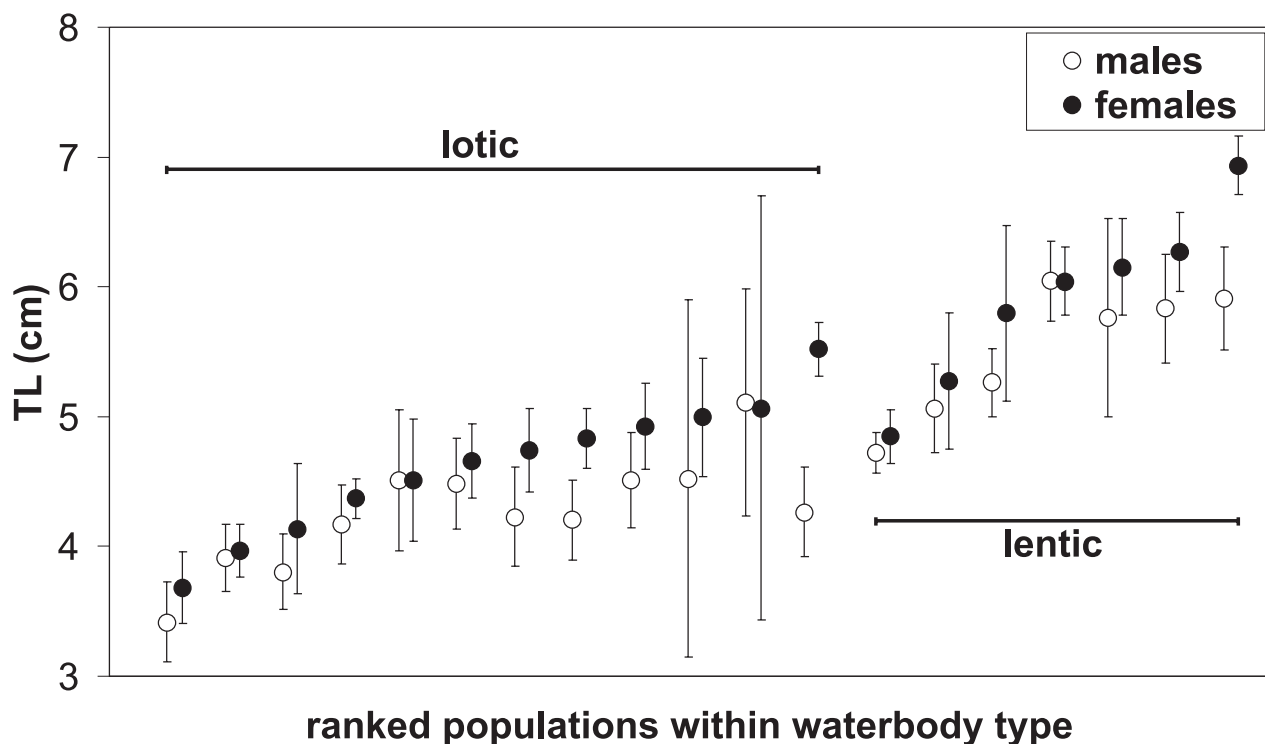
vary in its expression degree among snails from different sources (Estebenet and Martín, 2003). Adult size dimorphism varied also among egg masses collected from the same lake (Estebenet and Cazzaniga, 1998), presumably spawned by different females. Bigger female sizes have also been reported for other species of ampullariids (Burky, 1974; Keawjam, 1987; Lum-Kong and Kenny, 1989; Perera and Walls, 1996).

Shell thickness in *Pomacea glauca* (Linné, 1758) and *P. canaliculata* is inversely related to growth rate (Zischke *et al.*, 1970; Estebenet and Martín, 2003). The shell weight-shell length relationship studied in a temperate pond population of *P. canaliculata* did not adjust to a simple allometric model due to the ample variation of shell weights for snails of the same size, probably resulting from the different growth rates of snails born in different seasons (Estebenet, 1998).

Cazzaniga (1990) reported that male shells were significantly heavier than those of females of the same length for a sample from an artificial pond in Buenos Aires city. He suggested that female investment in egg-shell, and also in the perivitelline fluid (Turner and McCabe, 1990), is responsible of this difference. However, as in the case of shell length, the intersex differ-

ences in shell weight seem to be less pronounced in natural populations than in laboratory populations from the same sources (Estebenet and Martín, 2003).

The interpopulation variation in shell size and weight is very important, even within the same drainage basin, and it is basically ecophenotypic in origin since field differences disappeared when the snails were reared under homogeneous conditions (Martín and Estebenet, 2002; Estebenet and Martín, 2003). However, the precise influence of different environmental factors on these variables remains unclear. Cazzaniga (1987) proposed that snails inhabiting lentic habitats with soft bottoms have thinner and larger shells than those dwelling in habitats with hard bottoms and running waters. However, for a set of nineteen populations from Southern Buenos Aires province, shell length in both sexes was higher in lentic waterbodies than in lotic ones (Fig. 3), though no differences in shell weight were detected. Snails inhabiting lakes seem to be longer-lived than those from streams in this semiarid region, probably due to the highly variable hydrological regime of the latter (Martín and Estebenet, 2002). Females and males from lakes and reservoirs with hard bottoms showed lighter shells than those from shallow lakes with



**FIGURE 3.** Total shell length (TL, mean  $\pm$  95%CI) for males and females from lentic and lotic waterbodies in Southern Buenos Aires province ( $n \geq 30$  snails, except for five populations where  $n = 8, 11, 19, 21$  and  $21$ ).



sandy bottoms; female shells were also larger in the latter. The same pattern was observed among lotic waterbodies: males and females from sand-muddy bottoms showed heavier shells than those from limestone bottoms, while shell length was higher only for females from soft bottoms. Male growth rate shows less ecophenotypic plasticity than female's (Estoy *et al.*, 2002) and this would be related to the lesser degree of male size interhabitat variation.

### Operculum shape and weight

The length and width of the operculum grow with negative and positive allometry, respectively, resulting in an ontogenetic rounding of the operculum (Estebenet, 1998). At the same time the operculum becomes relatively thicker during growth. There seems to be some geographic, or at least interpopulation variation in the operculum growth patterns since, according to Guedes *et al.* (1981), the growth in length is isometric relative to the growth in width in a lentic population from Southern Brazil.

The growth rates of the length and specially of the width of the operculum are higher in males than in females (Estebenet, 1998) probably resulting in the sexually dimorphic operculum shapes reported by Cazzaniga (1990) in fully grown snails (i.e. masculine wider than feminine ones). However, the male opercula are lighter than those of females for a given shell size (Estebenet, 1998), probably due to a lesser thickness in the fore. On the other hand, Schnorbach (1995) reported that the opercula of females are concave while those of males are convex. In fact, we observed that the opercula of newborn and juveniles of both sexes are concave; the female operculum conserves this shape during the entire lifespan, while that of males becomes progressively convex in the labral fringe during maturity and thereafter, remaining concave in the rest of the surface.

### Final comments

The large conchological variation in *P. canaliculata* has been considered a serious hindrance to the study of several aspects of its biology. However, this apparently chaotic variation can be split in several biologically meaningful components, becoming an interesting subject of research on its own merit. In spite of the fact that many aspects of the conchological variation have been already studied, the available information includes, in

most cases, only one or a few populations from a restricted geographical region. The knowledge is even more limited for other species of *Pomacea* or other genera of apple snails, preventing the development of a comparative approach in conchological aspects at generic and familiar levels.

### Acknowledgements

We want to express our gratitude to Alicia Miravalles and Patricia Leonardi (Laboratorio de Ficología y Micología, UNS) for assistance in preparation of SEM specimens. This work was funded with grants by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, PEI 6067/04 and PIP 6150/05) and UNS (Universidad Nacional del Sur, PGI 24/B075 and PGI 24/B108). SB is a predoctoral fellow in CONICET. Alejandra Estebenet was a researcher in CONICET.

### References

- Asami T, Cowie RH, Ohbayashi K (1998). Evolution of mirror images by sexually asymmetric mating behavior in hermaphroditic snails. *Am Nat*, 152: 225-236.
- Berthold T (1991). Vergleichende Anatomie, Phylogenie und Historische Biogeographie der Ampullariidae (Mollusca: Gastropoda). *Abhand Naturwiss Vereins Hamburg (NF)*, 29: 1-256.
- Burky A (1974). Growth and biomass production of an amphibious snail, *Pomacea urceus* (Müller), from the Venezuelan Savannah. *Proc Malac Soc Lond*, 41: 127-143.
- Castellanos ZJ, Fernández D (1976). Mollusca Gasteropoda Ampullariidae. In: Fauna de agua dulce de la República Argentina. Ed Ringuelet RA, Fecic, Buenos Aires, pp. 1-33.
- Castro-Vazquez A, Albrecht EA, Vega IA, Koch E, Gamarra-Luques C (2002). Pigmented corpuscles in the midgut gland of *Pomacea canaliculata* and other neotropical apple-snails (Prosobranchia, Ampullariidae): a possible symbiotic association. *Biocell*, 26: 101-109.
- Catalán NMY, Fernández SN, Winik BC (2002). Oviductal structure and provision of egg envelopes in the apple snail *Pomacea canaliculata* (Gastropoda, Prosobranchia, Ampullariidae). *Biocell*, 26: 91-100.
- Cazzaniga NJ (1987). *Pomacea canaliculata* (Lamarck, 1801) en Catamarca (Argentina) y un comentario sobre *Ampullaria catamarcensis* Sowerby, 1874 (Gastropoda, Ampullariidae). *Iheringia, Série Zoologia*, 66: 43-68.
- Cazzaniga NJ (1990). Sexual dimorphism in *Pomacea canaliculata*. *Veliger* 33: 390-394.
- Cazzaniga NJ (2002). Old species and new concepts in the taxonomy of *Pomacea* (Gastropoda, Ampullariidae). *Biocell*, 26: 71-81.
- Cazzaniga NJ, Estebenet AL (1990). A sinistral *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Malacol Rev*, 23: 99-102.

- Cowie RH (2002). Apple snails as agricultural pests: the biology, impacts, and management. In: Molluscs as crop pests. Ed Barker GM, CABI, Wallingford, pp. 1-28.
- Demian ES, Ibrahim AM (1972). Sexual dimorphism and sex ratio in the snail *Marisa cornuarietis* (L.). Bull Zool Soc Egypt, 24: 52-63.
- Dillon RT (2003). The inheritance of *golden*, a shell color variant of *Marisa cornuarietis*. Malacol Rev, 31/32: 155-157.
- d'Orbigny AD (1835-1846). Voyage dans l'Amérique Méridionale. Tome V, Mollusques, XXLIH +711 pp. Chez P Bertrand, Paris.
- Estebenet AL (1998). Allometric growth and insight on sexual dimorphism in *Pomacea canaliculata* (Gastropoda: Ampullariidae). Malacologia, 39: 207-213.
- Estebenet AL, Cazzaniga NJ (1993). Egg variability and the reproductive strategy of *Pomacea canaliculata* (Gastropoda: Ampullariidae). Apex, 8: 129-138.
- Estebenet AL, Martín PR (2002). *Pomacea canaliculata* (Gastropoda: Ampullariidae): Life-history traits and their plasticity. Biocell, 26: 83-89.
- Estebenet AL, Cazzaniga NJ (1998). Sex-related differential growth in *Pomacea canaliculata* (Gastropoda: Ampullariidae). J Moll Stud 64: 119-123.
- Estebenet AL, Martín PR (2003). Shell interpopulation variation and its origin in *Pomacea canaliculata* (Gastropoda: Ampullariidae) from Southern Pampas, Argentina. J Moll Stud, 69: 301-310.
- Estoy GF, Yusa Y, Wada T, Sakurai H, Tsuchida K (2002). Size and age at first copulation and spawning of the apple snail, *Pomacea canaliculata* (Gastropoda: Ampullariidae). Applied Entomol Zool, 37: 199-206.
- Guedes LM, Fiori AM, Diefenbach CO (1981). Biomass estimation from weight and linear parameters in the apple snail *Ampullaria canaliculata* (Gastropoda: Prosobranchia). Comp Biochem Physiol, 68: 285-288.
- Heras H, Pollero RJ (2002). Lipoproteins from plasma and perivelline fluid of the apple snail *Pomacea canaliculata*. Biocell, 26: 111-118.
- Hylton-Scott MI (1958). Estudio morfológico y taxonómico de los ampulláridos de la República Argentina. Rev Mus Arg Cs Nat "B. Rivadavia", Zool, 3: 233-333.
- Jokinen EH (1984) Periostracal morphology of viviparid snail shells. Trans Am Microsc Soc, 103: 312-316.
- Keawjam RS (1987). The apple snails of Thailand: Aspects of comparative anatomy. Malacol Rev, 20: 69-90.
- Lum Kong A, Kenny JS (1989). The reproductive biology of the ampullariid snail *Pomacea urceus* (Müller). J Moll Stud, 55: 53-66.
- Martín PR, Estebenet AL (2002). Inter-population variation of life-history traits in *Pomacea canaliculata* (Gastropoda: Ampullariidae) in Southwestern Buenos Aires Province, Argentina. Malacologia, 44: 153-163.
- Martín SM (1984). Contribución al conocimiento de la familia Ampullariidae (Mollusca: Gastropoda) en el Río de la Plata. Tesis Doctoral, FCNyM, UNLP, La Plata, 149 pp.
- Perera G, Walls GH (1996). Apple Snails in the Aquarium. T.F.H. Publications, Inc., Neptune City. 121 pp.
- Raut SK, Aditya G (1999). Occurrence of Golden Mystery snail *Pomacea bridgesi* (Gastropoda: Ampullariidae) in West Bengal, India. Curr Sci, 77: 1389-1390.
- Ribi G, Oertli S (2000). Frequency of interespecific matings and of hybrid offspring in sympatric populations of *Viviparus ater* and *V. contectus* (Mollusca: Prosobranchia). Biol J Linn Soc, 71: 133-143.
- Schnorbach HJ (1995). The golden apple snail (*Pomacea canaliculata* Lamarck) an increasingly important pest in rice, and methods of control with Bayluscid®. Pflanzenschutz-Nachrichten Bayer, 48: 313-346.
- Tanaka K, Watanabe T, Higuchi H, Miyamoto K, Yusa Y, Kiyonaga T, Kiyota H, Suzuki Y, Wada T (1999). Density dependent growth and reproduction of the apple snail, *Pomacea canaliculata*: a density manipulation experiment in a paddy field. Res Popul Ecol, 41: 253-262.
- Turner R, McCabe C (1990). Calcium source for protoconch formation in the Florida apple snail, *Pomacea paludosa* (Prosobranchia: Pilidae): more evidence for physiologic plasticity in the evolution of terrestrial eggs. Veliger, 33: 185-189.
- Wada T (1997). Introduction of the apple snail *Pomacea canaliculata* and its impact on rice agriculture. Proceedings of the International Workshop on Biological Invasions of Ecosystems by Pests and Beneficial Organisms, pp. 170-180, Tsukuba.
- Yusa Y (2004). Inheritance of colour polymorphism and the pattern of sperm competition in the apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae). J Moll Stud, 70: 43-48.
- Zischke JA, Watabe N, Wilbur K (1970). Studies on shell formation: measurement of growth in the gastropod *Ampullarius glaucus*. Malacologia, 10: 423-439.

