



## ECOSYSTEMS

# Sexual dimorphism in shell shape: is *Pomacea canaliculata* an exception or an example among Neotropical apple snails?

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**Abstract:** Sexual dimorphism has often been recorded in apple snails (Caenogastropoda Ampullariidae), but reports are concentrated in a few species, either invasive or with biocontrol potential, which suggests some taxonomic bias. To find out evolutionary and ecological correlates of sexual dimorphism it is necessary to detect and quantify it but also is important to detect its absence. Our aims were to confirm or not the existence of sexual dimorphism in shell shape of *Felipponea neritiformis* and *Asolene platae*, using *Pomacea canaliculata* as reference and applying the same methodology (landmark-based geometric morphometrics) and statistical power. Significant intersexual differences were only found in *P. canaliculata* and, in a lesser degree, in *F. neritiformis*: males have larger apertures relative to body whorl and more rounded apertural outer edges than females. Female shells are larger in *F. neritiformis* and *P. canaliculata*, but not in *A. platae*. Using comparable methodologies and statistical power, sexual dimorphism in shell shape is detectable in some apple snails but not in others. Interspecific variation in sexual dimorphism in the Ampullariidae is not only due to taxonomic bias and deserves more research to establish the main patterns and possible causes.

**Key words:** Ampullariidae, geometric morphometrics, *Marisa*, *Asolene*, *Felipponea*.

## INTRODUCTION

Differences between sexes in the average of certain traits are termed sexual dimorphism and they are especially interesting when occurring in traits not directly related to reproductive function because of the diverse ways in which these secondary sexually dimorphic traits increase mating or reproductive success (Fairbairn 2016). Sexual dimorphism has attracted scientific interest since the beginning of the evolution theory. Darwin and Wallace proposed two different mechanisms for the origin of the sexual dimorphism based on sexual selection and ecological differences between sexes, respectively (Slatkin 1984, Hedrick & Temeles 1989, Punzalan & Hosken 2010). Current theoretical debate turns around two competing,

but not mutually exclusive, paradigms (De Lisle 2019): the Darwin-Bateson's paradigm, asserting that different sex-specific optima have an ultimate evolutionary cause in anisogamy, and the Ecological Character Displacement paradigm, asserting that intersexual competition generates disruptive selection on traits related to resource acquisition. Most animal species are dioecious and exhibit some form of externally apparent sexual dimorphism although prevalence among clades varies substantially (Fairbairn 2016). Although mollusks are the second phyla regarding species diversity, they have been absent at the debate (De Lisle 2019). Among mollusks, groups with external fertilization rarely exhibit sexual dimorphism, while those that have internal fertilization, like some gastropods

and cephalopods, show sexual dimorphism (Levitan 1998). According to some authors, sexual dimorphism is infrequent in mollusks in general (Mori et al. 2017) and gastropods in particular (Wilbur & Yonge 1964). Whether these statements reflect true patterns of sexual dimorphism in these groups remains largely unknown, the lack of studies aimed specifically to address this subject being a possibility. For instance, external sexual dimorphism was known in only three genera among terrestrial Caenogastropoda but a marked size dimorphism has been recently reported for two other genera (Páll-Gergely et al. 2020).

Though often unacknowledged, sexual dimorphism is an important component of biodiversity (Tsuji & Fukami 2020) and information about its occurrence (or not) is necessary for a sound understanding of ecology, behavior and life history of a species (Kitano et al. 2007). The Ampullariidae is a highly diverse family of large freshwater Caenogastropods from America, Africa and Asia in which sexual dimorphism has been often recorded and reviewed (Estebenet et al. 2006, Hayes et al. 2015). A species in particular, *Pomacea canaliculata* (Lamarck 1822), is highly dimorphic regarding size, shell and operculum shape (Cazzaniga 1990, Estebenet & Cazzaniga 1998, Estebenet et al. 2006, Tamburi & Martín 2013, 2012). This species also exhibits marked sexual dimorphism in ecophysiological and behavioral traits: feeding rates and efficiencies (Tamburi & Martín 2009a), growth rates (Estebenet & Martín 2003) reaction norms to food availability (Estoy et al. 2002, Tamburi & Martín 2009b), predation risk (Guo et al. 2017, 2019b), predator avoidance behavior (Xu et al. 2014), cold and desiccation tolerance (Guo et al. 2019a), etc. The diversity of dimorphic traits is perhaps not surprising because it is by far the most studied species within the Ampullariidae family due to its invasiveness and

the multiple impacts that it has provoked in SE Asia (Hayes et al. 2015, Horgan et al. 2014, Martín et al. 2019). Reports of sexual dimorphism in the other eight genera of the family are scanty and sexual dimorphism has seldom been studied purposely. Detection of sexual dimorphism in shell shape in *Marisa cornuarietis* (Linnaeus 1758) was also linked to bioecological studies related to its use in biological control (Demian & Ibrahim 1972). These antecedents indicate that there is probably a strong taxonomic bias in the knowledge of sexual dimorphism within this family, driven in part by applied interests focused on a few species.

Sexual dimorphism in shell size is relatively easy to detect, even in museum material, and so it has been often reported in Caenogastropoda (Collin 2018). On the other hand, sexual dimorphism in shell shape in this clade requires statistical methods to detect it (Páll-Gergely et al. 2020). Classical tests based on linear distances and multivariate analyses may not be sensitive enough in some cases and advanced methods like geometric morphometrics (GM) are required (Minton & Wang 2011). Sexual dimorphism in shell shape may arise also as a result of the combination of allometry and sexual dimorphism in size which would require GM techniques to disentangle the two factors (Rohlf 1993, Klingenberg 2010). The diversity of methods to investigate sexual dimorphism in shape also conspires against the recognition of its patterns. The patterns of sexual dimorphism may be further distorted by publication bias against non-significant differences between sexes (Jennions & Møller 2002) and by a sampling bias against subtle examples of sexual dimorphism (Bonduriansky 2007). This would make sexually dimorphic cases more frequent relative to total published studies than in fact are and perhaps would conceal that sexual dimorphism may be indeed exceptional in some

taxa. To find out evolutionary and ecological correlates of sexual dimorphism it is necessary to detect and quantify it but also is important to detect its absence with a certain level of statistical power.

Though sharply escalating in *Pomacea* spp., bioecological information remains almost null for species of other genera of Neotropical apple snails (Hayes et al. 2015) in which sexual dimorphism in shell shape has not been addressed or published yet (e.g. Schilithz 2013). In the Neotropical genus *Marisa* Gray 1824 some degree of sexual dimorphism has been reported (Demian & Ibrahim 1972) but for comparative purposes it has the inconvenient of having a planispiral shell. The aims of the present study were to confirm the existence or not of sexual dimorphism in shell shape of two Neotropical apple snails from different genera (*Felipponea neritiformis* Dall 1919 and *Asolene platae* (Maton 1811)) using a well-known example (*P. canaliculata*) as a reference and applying the same methodology and statistical power for all three cases. Sexual dimorphism in size is also reported for *F. neritiformis* since almost no quantitative information is available for the genus.

### Abbreviations

GM geometric morphometrics.

LM landmark.

OAE outer apertural edge.

SAV Shell apertural view.

SLM sliding semi-landmark.

## MATERIALS AND METHODS

### Origin of the snails

The set of *P. canaliculata* shells used here as reference of statistical power was previously studied by Tamburi & Martín (2013, 2012, 2009b), showing sexual dimorphism in size, in

the shape of the aperture and the globosity of the shell. This snail shells were obtained from a single egg mass laid by a laboratory female that copulated with only one male to minimize genetic variation; both parents were collected from Curamalal stream (36°57'36"S, 62°9'31"W, Buenos Aires province, Argentina; Tamburi & Martín 2009a). The snails used here were reared until sexual maturity at five different food availability regimens (from 100 to 47%; Tamburi & Martín 2009a, b).

*Asolene platae* hatchlings were obtained from egg masses laid by snails collected in 2008 from Regatas Lake (34°33'36"S, 58°26'05"W, Buenos Aires City, Argentina) and raised in the laboratory to study the life cycle and the demographic parameters (Tiecher et al. 2016, 2014). The shells used here all belong to mature snails that died naturally during a four years cohort study.

A sample of 75 adult snails of *F. neritiformis* was collected in 2014 at Bonito Stream (27°26'36"S, 54°56'02"W, Misiones Province, Argentina) and maintained in laboratory conditions (fed with lettuce *ad libitum*; in a rearing room with 25±2°C and 14/10h light/dark) for a few days for behavioral observations and then frozen and maintained at -20°C until processing.

All the snails were collected with the required legal collection permissions. Voucher specimens of the three species were deposited in the National Invertebrate Collection at Museo Argentino de Ciencias Naturales (MACN): *P. canaliculata* (MACN-In: 43436), *A. platae* (MACN-In: 43435) and *F. neritiformis* (MACN-In: 43434).

### Sexing of the snails

The snails were sexed according to their copulatory behavior or egg laying in the case of *A. platae* (Tiecher et al. 2016) and by the observation of the testicle through the translucent shell and the concave operculum in the case of

*P. canaliculata* (Tamburi & Martín 2009a, b). After their natural death (*A. platae*) or their sacrifice (*P. canaliculata*) the snail bodies were extracted from shell and the sex was confirmed by the presence of a penis sheath and testicle in males and the presence of an albumen-capsule gland-complex in females (Cadierno et al. 2018).

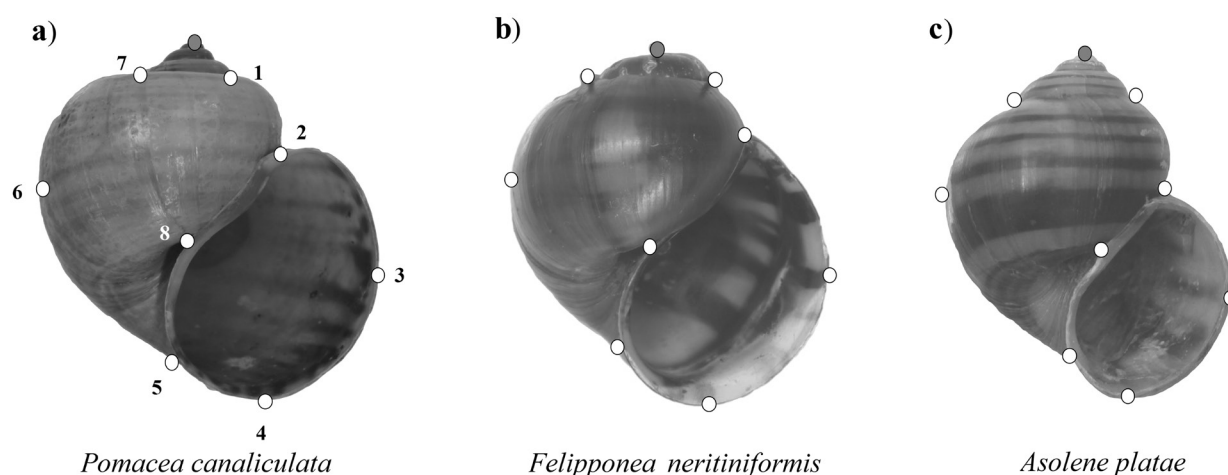
In the case of *F. neritiformis* observations of a few copulating pairs made in the field and in the laboratory suggested that the outer lip of the shell aperture is much more rounded in males than in females in which it is an almost straight line. To check if this dimorphism in the shape of the outer lip of *F. neritiformis* was in fact sex related and if it could be a reliable method to visually differentiate males from females, two authors (SB and MJT) tentatively sexed the all the snails using it. After this, the snails' soft parts were extracted from the shell and the sex of the snails was anatomically confirmed by dissection as in the two other species. A Chi-squared test was performed to compare the sex assigned by the authors with the sex attributed by anatomical dissection.

### Shell size analysis

To explore the possibility of sexual size dimorphism in *F. neritiformis* the shell length (SL, mm) of 23 males and 52 females was measured with a caliper ( $\pm 0.05$  mm), as the distance between the farthest points of the spire and aperture and parallel to the columellar axis. For *P. canaliculata* and *A. platae* this information was already published in Tamburi & Martín (2009a) and in Tiecher et al. (2016).

### Shell shape analysis

Two different perspectives of the shells were analyzed through GM to study sexual dimorphism in the three species: a classical shell apertural view (SAV, Fig. 1) and the shape of the outer apertural edge in dorsal view (OAE, Fig. 2), as suggested by the observations in *F. neritiformis*. The snails shells of each of the three species were systematically photographed in both perspectives (SAV and OAE) using a Canon T3i digital reflex camera with a 55 mm Helios lens. To study the degree of sexual dimorphism in *F. neritiformis* and *A. platae* with a similar statistical power than in *P. canaliculata* almost



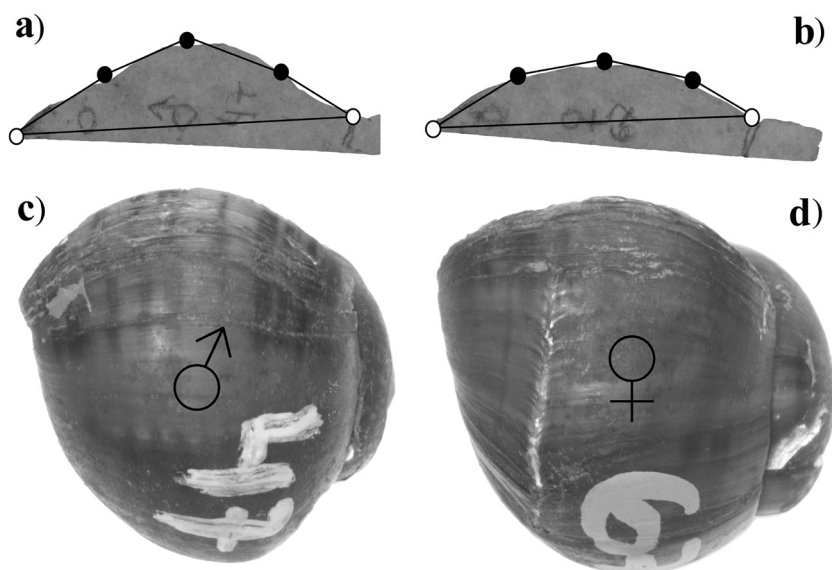
**Figure 1.** Landmarks studied (LM, white dots) in the apertural view (SAV) of the a) *Pomacea canaliculata*, b) *Felipponea neritiformis* and c) *Asolene platae*. Grey dots mark the apex of the shells and were used to align the position of the snail in the photographs, although were not used as landmarks in the GM analysis. The pictures were re-scaled to show the same shell length (distance from the apex to landmark 4).

the same number of snails was used in each species (*ca.* 40) with a balanced distribution of males and females. The number of snails used in each species and sex varied a little depending on the integrity of the shell. For the morphological analyses of SAV 40 shells of *P. canaliculata* (20 males and 20 females), 44 of *F. neritiformis* (23 males and 21 females) and 47 of *A. platae* (22 males and 25 females) were used. For the morphological analyses of OAE 38 shells of *P. canaliculata* (20 males and 18 females), 45 of *F. neritiformis* (24 males and 21 females) and 42 of *A. platae* (22 males and 20 females) were studied.

Following the GM methodology that previously found sexual dimorphism in the SAV of *P. canaliculata* (detailed by Tamburi & Martín 2013) eight landmarks (LM) were digitized using TPSdig2 software (version 2.3; Rohlf 2017). The *F. neritiformis* shells collected from field have usually an eroded apex, which may involve the loss of the first whorls (Fig. 1), and therefore can vary strongly in length. As a consequence, the apex was not considered as a reliable landmark in *F. neritiformis* and was not used altogether in *P. canaliculata* and *A. platae* to maintain the

same number of landmarks and consequently a comparable statistical power. The photographs could be aligned according to the columellar axis by marking with a pin the projection of the columellar axis (Fig. 1). Landmarks 1 (LM 1) and 7 (LM 7) are right and left intersections of the last complete suture in the shell contour; LM 2 is the upper suture between the body whorl and the aperture; LM 3 is the most external point of the aperture on the right border and LM 6 is the most external point of the body whorl on the left border of the shell; LM 4 is the extreme point of the aperture border opposite to the apex; LM 5 is the intersection of the aperture border and the outline of the body whorl; LM 8 is the intersection of the umbilicus border and the aperture border. In *F. neritiformis* was also necessary to mark the right (LM 1) and left (LM 7) intersections of the last complete suture in the shell outline with pins because it is often hidden by the body whorl.

To analyze in detail the sexual dimorphism in OAE for the three studied species GM techniques were used. The shape of the aperture was traced with a masking tape, then was cut and pasted in a sheet of paper for posterior analysis (Fig.



**Figure 2.** Technique used to study the shape of the outer aperture edge (OAE) in apple snail shells. The edge of the aperture was traced with a masking tape (a and b), then photographed and used to set two landmarks (LM; white dots) and three semilandmarks (SLM; black dots). The photographs correspond to the male (a and c) and the female (b and d) of *Felipponea neritiformis* that showed the greatest differences in the discriminant analyses (see Results).



2). The contour drawn in the masking tape was photographed and used to set two landmarks and three sliding semi-landmarks (SLM). LM1 is the umbilicus (corresponding to LM 8 in SAV; Fig. 1), LM2 is the suture between the body whorl and the aperture (corresponding to LM 2 in SAV; Fig. 1). Sliding semi-landmarks were generated by drawing curves along the edge outline between LM1 and LM2 and then using the TPSdig2 (Rohlf 2017) resampling tool to reduce the number of points to three equidistant SLM.

The position of the SLM was relaxed with TPSrelw software (version 1.69; Rohlf 2015) using the technique of minimizes the bending energy matrix. The same software was used to perform the Procrustes superimposition to correct differences in scale, position and orientation. The aligned specimens (shape) and the centroid size were studied in detail with MorphoJ Software (Version 1.06b; Klingenberg 2011).

MorphoJ Software was used to perform the Procrustes superimposition of images of the two perspectives (SAV and OAE) to account for differences in position, orientation and scale and, afterwards, to study in detail the aligned images (shape) and centroid size (size). The centroid size is a measure of size and is calculated by the software as the square root of the total quadratic distance between each LM and the centroid of all LM in a particular specimen (Bookstein 1991). Wireframe graphs were drawn connecting specific LM with straight lines to visualize easily the main differences in shape along each analysis. The polygon traced by LM 1, 2, 8, 5, 6 and 7 delimits the body whorl whereas LM 2, 3, 4, 5 and 8 delimit the shell aperture.

Discriminant analysis was performed to seek for sexual dimorphism in each species separately. Before this procedure, the significance of the allometry was tested following the procedure detailed in Viscosi & Cardini (2011) for each

sex of each species. Multivariate regressions of shape onto centroid size were performed for each group. As it was no significant allometry (see Results) in any of the six groups (sex by species), a discriminant analysis without the allometry correction provided by MANCOVA was performed. The reliability of the discrimination was assessed by a “leave-one-out” cross-validation technique provided by MorphoJ Software and a permutational t-test.

## RESULTS

### Shell size analysis

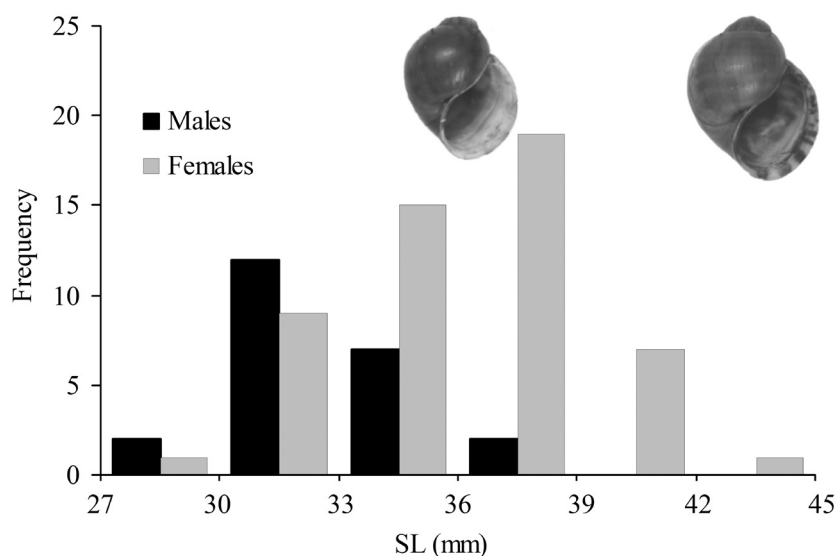
The mean shell length of *Felipponea neritiformis* females was larger in females than in males (means: 35.61 and 32.77 mm, respectively; Welch test:  $F=22.79$ ,  $p<0.01m$ ). The largest size reached by a female was 42.3 mm whereas in the case of males it was 37.3 mm (Fig. 3).

Using the criterion of the roundness of the outer lip for visually sexing *F. neritiformis*, the sex of the snails was successfully determined in 93.4% of the cases, far away from the 50% expected by chance ( $X^2=57.36$ ;  $p<0.001$ ). The percentage of success was similar for males and females (91.7 and 94.2%, respectively).

### Shell shape analysis

Allometry in the SAV shape was not detected in males or females of *P. canaliculata*, *F. neritiformis* or *A. platae*. In *P. canaliculata*, allometry explains a non-significant 8.76% of the morphological variation ( $p=0.0958$ ) in the females and a 9.36% ( $p=0.1137$ ) in the males. In *F. neritiformis* the corresponding values were 7.63% ( $p=0.1550$ ) for females and 4.52% ( $p=0.4288$ ) for males whereas in *A. platae* they were 3.10% ( $p=0.5991$ ) and 6.83% ( $p=0.1785$ ), respectively.

The discriminant functions (Fig. 4) reached a 100% of correct classification of the sex of the



**Figure 3. Sexual dimorphism in *Felipponea neritiformis*. Frequency distributions of shell length (SL) for 23 males and 52 females. The shell on the left is the largest male (37.3 mm) and the one on the right is the largest female (42.3 mm).**

specimens by cross-validation in *P. canaliculata* (Procrustes distance=0.068;  $p<0.001$ ), whereas the corresponding values were 75% in *F. neritiformis* (Procrustes distance=0.028;  $p=0.016$ ) and 66% in *A. platae* (Procrustes distance=0.0218;  $p=0.119$ ). In *P. canaliculata* female shells were more globose with an expanded body whorl and a relatively smaller and rounder aperture than males. The same dimorphism was noticeable in *F. neritiformis* although with a smaller magnitude and it was practically imperceptible in the case of *A. platae*.

Allometry in the GM analysis of OAE showed that in *P. canaliculata* only the 11.4% of the morphological variation ( $p=0.144$ ) was explained by size in females and a 0.234% ( $p=0.9695$ ) in the case of males. Similar results were observed for *F. neritiformis* (females: 0.79%,  $p=0.8781$ ; males: 0.548%,  $p=0.9177$ ) and for *A. platae* (females: 2.21%,  $p=0.6537$ ; males: 1.46%,  $p=0.7273$ ).

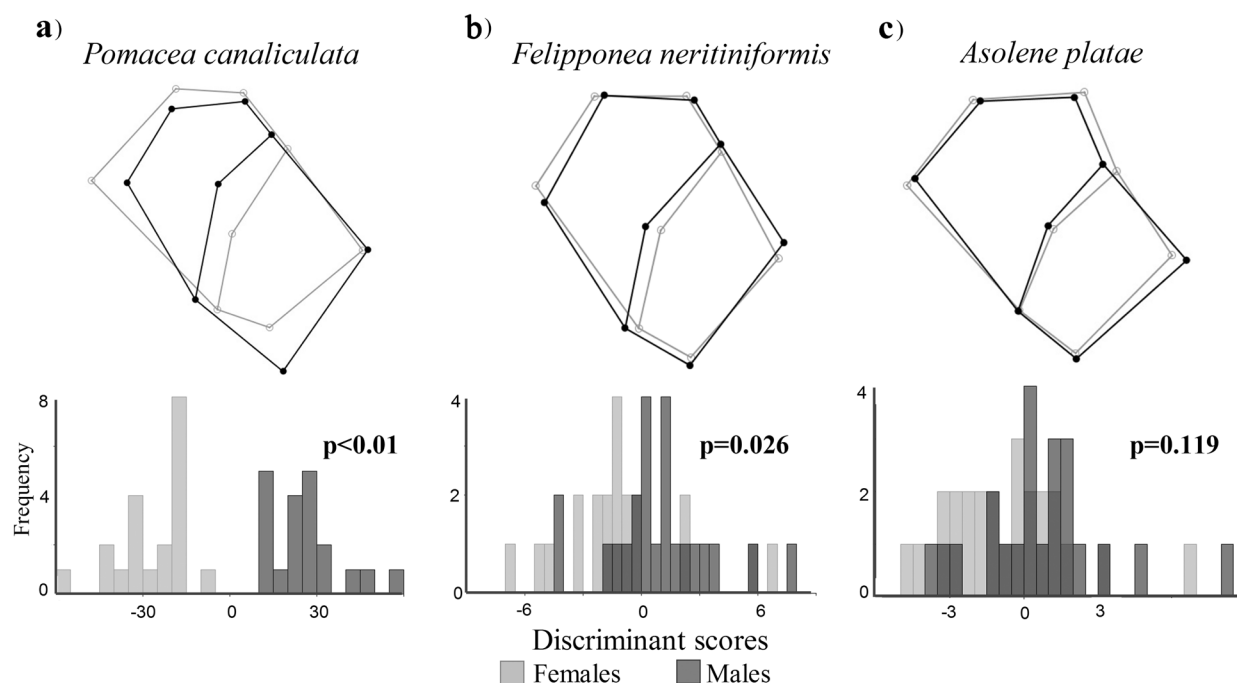
In the GM analysis of OAE from *P. canaliculata* (Fig. 5) the discriminant function allowed to classify correctly the sex of a 89.5% of the snails through “leave-one-out” cross-validation method (Procrustes distance=0.0589;  $p<0.001$ ). This method reached a 66.67% of correct classification by cross-validation (Procrustes

distance=0.0508;  $p<0.001$ ) in the case of *F. neritiformis* and a 50% of correct classification by cross-validation (Procrustes distance=0.0173;  $p=0.692$ ) in the case of *A. platae*. The shape of the OAE is more rounded and protruded in males than in females. This is very clear in the case of *P. canaliculata*, intermediate in the case of *F. neritiformis* and is not distinguishable in the case of *A. platae*.

## DISCUSSION

Our study showed that using the same methods and the same statistical power, sexual dimorphism in shell shape is revealed in some apple snails but not in others, indicating that variation in occurrence of sexual dimorphism is not only due to lack of specific studies in some taxa. The GM technique found intersexual shell shape differences in *P. canaliculata* and *F. neritiformis* but not in *A. platae*.

The discriminant functions showed intersexual differences in SAV in two out of three analyzed species. In *P. canaliculata* this intersexual difference was previously described as a more rounded and larger aperture relative to body whorl in males (Tamburi & Martín 2013) and



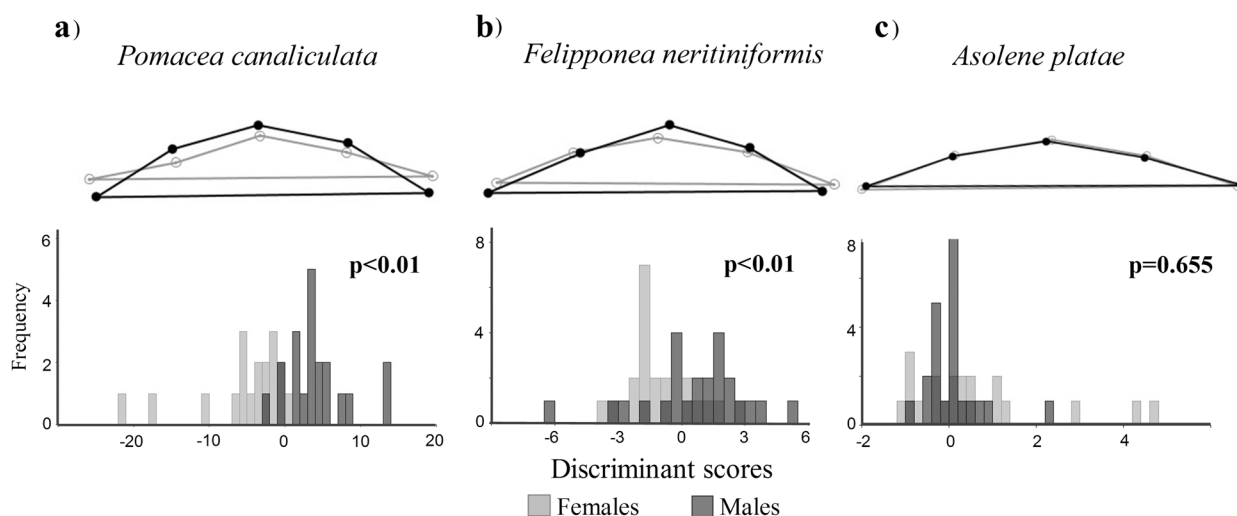
**Figure 4.** Geometric morphometrics analyses of intersexual differences in the shell apertural view (SAV) of a) *Pomacea canaliculata*, b) *Felipponea neritiformis* and c) *Asolene platae*. Wireframe graphs represent the shape deformations that differentiate females (gray) and males (black); scale factor applied to wireframes deformation was 4 in all species. Bars graph represent the scores of the discriminant function using a “leave-one-out” cross-validation method.

the same characterization of sexual dimorphism seems to be valid for *F. neritiformis*. Good values of correct sex identification were obtained for *F. neritiformis* while in the case of *A. platae* the differences were non-significant, which is consistent with results obtained for two other species of *Asolene* (Schilithz 2013). This sexual dimorphism is pronounced and may be used to determine the sex of *P. canaliculata* but it is probably unreliable for the other two species. On the other hand, the outer lip of *F. neritiformis* aperture allows external differentiation of males and females by observing them with the naked eye. The GM technique applied to the OAE could also be used to determine the sex although with lesser degree of confidence, probably due to the low number of LM and SLM used or to the fact that during sexing operators turn the shell to maximize the outer lip protrusion in each snail. Surprisingly, the present results showed that

in the case of *P. canaliculata*, the most studied species in this group, this method differentiates the sex of the snails with even higher success than in *F. neritiformis*. The high degree of sexual dimorphism in the aperture view of *P. canaliculata* shells probably attracted much more visual attention in previous studies and led to neglect the differences in the shape of the shell aperture edge.

Sexual dimorphism in shells of Caenogastropoda appears to be related to female fecundity and reproductive functions (Collin 2018). Shell shape sexual dimorphism has been related to brooding in several gastropods, usually involving an enlarged body whorl, aperture, umbilicus or adaperatural callus to support embryos, eggs or egg capsules (e.g. Lindberg & Dobberteen 1981, Minton & Wang 2011, Márquez & Averbuj 2017). In *P. canaliculata* and *F. neritiformis* the proportionally larger





**Figure 5.** Geometric morphometrics analyses to establish intersexual differences in the outer apertural edge (OAE) of a) *Pomacea canaliculata*, b) *Felipponea neritiformis* and c) *Asolene platae*. Wireframe graphs represent the shape deformations that differentiate females (gray) from males (black); scale factor applied to wireframe deformation was 2 in all species. Bar graphs represent the scores of the discriminant function using “leave-one-out” cross-validation method.

female body whorl relative to the aperture is probably related to the large size of their capsule-albumen glands complexes (Simone 2004) which, due to adaptations to produce calcareous eggshells (Catalán et al. 2002, Cadierno et al. 2018, Berthold 1991), are especially bulky in the more dimorphic *P. canaliculata*. On the contrary, the shell apertures are relatively wider in males and this is perhaps related to the great development of the penis sheath that arises from the mantle border and is folded over the gill in the mantle cavity. Perhaps the wider aperture allows an easier water flow around the penis sheath, which again is larger in the more dimorphic species (Berthold 1991).

Our study also revealed a new aspect of sexual dimorphism in the shell shape of Neotropical apple snails. The outer edge of the aperture is more rounded and protruded in males than in females, and again this dimorphism is stronger in *P. canaliculata* than in *F. neritiformis* and non-significant in *A. platae*. A plausible explanation for stronger developed traits in males is as offensive weapons in male to

male competition for females, although evidence is lacking in the Caenogastropoda (Collin 2018). Considering the copulation position in apple snails (Burela & Martín 2009, Tiecher et al. 2014, Gurovich et al. 2017), a defensive function during copulation may be equally likely, as the protruded lip would cover the basis of the penis sheath while it is inserted in the mantle cavity of the female. Some support to this last hypothesis is that copulation duration is related to the degree of dimorphism in this trait: 12 to 20 hs in *P. canaliculata* (Burela & Martín 2011), 4 to 5 h in *F. neritiformis* (SB, unpublished data) and 2.66 hs in *A. platae* (Tiecher et al. 2014).

Transitions in reproductive strategies in Caenogastropoda may be reflected in their sexual dimorphism (Collin 2018). Perhaps the transition from underwater oviposition of gelatinous egg masses, as in *Asolene* and *Felipponea*, to aerial oviposition of eggs with calcareous eggshells in apple snails like *Pomacea* and *Pila* resulted in some enhancement of sexual dimorphism. In fact, Keawjam (1987) reported that in *Pila* spp. “males are generally less swollen and smaller

than the females” which indicates that their sexual dimorphism deserves a more systematic study.

As in most apple snails studied heretofore (Estebenet et al. 2006, Hayes et al. 2015), *F. neritiformis* females are larger than males. Size dimorphism in this species is strong, with 27 % of the females being larger than the largest male. Faraco et al. (2002) based on two males and two females of a species identified as *F. neritiformis* also stated that males were smaller than females. Sexual size dimorphism, especially when it is not very marked, may have been overlooked in many other species, as suggested by Páll-Gergely et al. (2020) for terrestrial Caenogastropoda. Laboratory studies indicate that in other apple snails, and perhaps in *F. neritiformis*, the proximate cause of size dimorphism is a higher juvenile growth rate and not an extended life span of females (Tiecher et al. 2016, Gurovich et al. 2018, Estebenet & Martín 2003). However, mortality rates may contribute to the smaller size of male apple snails in natural habitats, as the negative effects of cold and desiccation stress (Guo et al. 2019a), predation risk (Xu et al. 2014) and food deprivation (Tamburi & Martín 2016) are all higher in them.

Rensch’s rule states that in clades where males are larger size dimorphism increases with size whereas decreases with size in those where females are larger (Fairbairn 2016). Although data are still scanty, Neotropical apple snails clearly do not comply with Rensch’s rule, the smallest species (*A. platae*, female SL: 25 mm) being almost monomorphic in size (7%; Tiecher et al. 2016). On the other hand, the large *Pomacea americanista* (Ihering 1919) (female SL: 62 mm) and *Pomacea urceus* (Müller 1774) (female SL: 117 mm) are more strongly dimorphic (asymptotic or maximum size of females 26 and 15% larger than those of males, respectively; Gurovich et al. 2018, Lum-Kong & Kenny 1989).

On practical grounds, the detection of external sexual dimorphism in apple snails opens the possibility to recognize the sexes without harmful, disturbing or lethal procedures, which would allow performing more detailed population viability studies, especially in apple snails that may be vulnerable (Tiecher et al. 2017, Gurovich et al. 2017, 2018). The ability to non-destructively sex apple snails at early ages (i.e. before reproductive behavior becomes apparent) has allowed many improvements in basic experimental research (Tamburi & Martín 2009b; Yusa & Kumagai 2018). In some cases, early sexing of apple snails may also result in a significant improvement of breeding technology for aquaculture (Ramnarine 2004) or conservation (Garr et al. 2012), but studies aimed to detect external sexually dimorphic traits in these species have not been published. It is worth mentioning that shell shape of *P. canaliculata* was not considered sexually dimorphic up to 1990 (Cazzaniga 1990).

Our study revealed that using comparable methodologies and statistical power, sexual dimorphism in shell shape is detectable in some apple snail species but not in others. The interspecies variation in this aspect of biodiversity, and also its interpopulation component (Estebenet & Martín 2003), may be high in apple snails and deserves more research to establish the main patterns and possible causes.

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Each author has contributed significantly to the intellectual content of the work. NET, SB and PRM: conceptualization, data sampling, statistical analyses and interpretation, writing, reviewing and editing. MJT: conceptualization, data sampling, statistical analyses and interpretation.

