Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 76(2) :199-211, abr./jun. 1981

BIOMPHALARIA OCCIDENTALIS SP. N. FROM SOUTH AMERICA (MOLLUSCA BASOMMATOPHORA PULMONATA)

W. LOBATO PARAENSE

A new species of South American planorbid snail, Biomphalaria occidentalis, *is described. It is indistinguishable from* B. tenagophila *(Orbigny, 1835), by the characteristics of the shell and of most organs of the genital system. In* B. tenagophila *there is a pouch on the ventral wall of the vagina (Fig. 4A, vp), absent in* B. occidentalis *(Fig. 3A), and the prepuce is much wider than the penial sheath, its width increasing distalward (Fig. 4, ps, pp), whereas in* B. occidentalis *the prepuce is wider than the penial sheath but keeps about the same width all along (Fig. 3, ps, pp).*

The two species are biologically separate by absolute reproductive isolation.

The geographical distribution of B. occidentalis *is shown in Fig. 14. So far it has been found in the Brazilian states of Acre, Amazonas (?), Mato Grosso, Mato Grosso do Sul and Paraná, and in Paraguay. Its type-locality is Campo Grande, state of Mato Grosso do Sul, where it was collected from several biotopes related to affluents of the Aquidauana river, chiefly Córrego Prosa and Córrego Ceroula.*

Specimens were deposited in the following malacological collections: Instituto Oswaldo Cruz, Rio de Janeiro; Academy of Natural Sciences, Philadelphia; Museum of Zoology, University of Michigan; and British Museum (Natural History).

In 1955, Paraense & Deslandes published a description of *Biomphalaria tenagophila* (under the synonymic denomination *Australorbis nigricans)* based on specimens collected at Itajubá, Minas Gerais state. Among the samples from other localities examined in the following years there appeared, here and there, some ones with certain anatomical characteristics somewhat departing from the mentioned description, the observed differences having been ascribed to intraspecific variation. Finally evidence accumulated showing that such different populations were more frequent on the western region of this country. Crossbreeding experiments showed that they belong to a species different from *Biomphalaria tenagophila* (Orbigny, 1835), to which I now propose the name *Biomphalaria occidentalis* sp. n.

Instituto Oswaldo Cruz, Caixa Postal 926 – 20000 – Rio de Janeiro – Brazil.

Supported by grant SIP/02-001, Programa do Trópico Úmido, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Received for publication on January 29th and accepted on March 30th, 1981.

MATERIAL AND METHODS

The present species was collected from 25 localities in the Brazilian states of Amazonas, Acre, Mato Grosso, Mato Grosso do Sul and Paraná, and in Asunción, Paraguay, numbered 113 to 137 on Fig. 14.

At least 10 specimens from each sample were preserved for anatomical study. To prevent retraction, they were placed in a 0.1% solution of nembutal in tap water for about 4 hr. They were then killed by plunging into hot water at 70°C for about 40-50 sec and transferred to cold water. Each specimen (under water) was gently pulled by the foot with a tweezer, so as to disconnect the attachment of the columellar muscle to the shell. The whole animal was drawn out of the shell and fixed in modified Railliet-Henry's fluid: distilled water 930 ml, sodium chloride 6 g, formalin 50 ml, acetic acid 20 ml.

The number of ovotestis diverticula was determined by fine dissection of every unit. The exact number of divisions of the diverticula is sometimes difficult to ascertain, for instance, when several diverticula converge so closely as to render impossible to individualize the stalks of one or two of them.

Examination of the vaginal region to determine the presence or absence of a vaginal pouch requires a careful preparation of the area. The vaginal wall must be rid of its covering membrane and connecting fibers which may disguise the underlying surface.

Crossing experiments were performed between specimens grown in isolation since hatching, and laying eggs regularly, using albinism as genetic marker (Paraense, 1955). A specimen of *B. occidentalis* (of which only melanic individuals have been found so far) was put in a small aquarium with an albino *B. tenagophila* of about the same size from Taubaté (São Paulo state), Joinville (Santa Catarina state) or Asunción (Paraguay), and left together for 10 days. They were then transferred to separate aquaria and observed for about 20 days. The snails deposited their egg masses on the underside of floating leaves (*Pistia stratiotes*), from which they were removed every morning to small petri dishes with aquarium water for subsequent examination under the stereomicroscope. The embryos were observed for identification of their melanic or albino character, indicated by the presence or absence of the eye-spots on the 5th day at 26-28°C. As albinism is recessive, the ocurrence or not of fertilization of the albino's eggs by the sperm of its melanic mate was ascertained merely by inspection of its F_1 offspring. As to the melanic mates, the eggs of which always develop into melanic embryos, the possibility of cross-fertilization was investigated by examining the F_2 embryos. For this purpose the first egg-masses laid by the melanic mate after separation of the couple were kept in small petri dishes, and the first 30 hatched snails (F_1s) were reared separately in small aquaria up to egg-laying. The egg-masses of 20 of these F_1s were observed as described above, at least 50 F_2 embryos from each F_1 being examined. As these self-fertilizing F_1s were functioning as a male-female couple, it was expected that their progeny showed a proportion of 3 melanic to 1 albino embryos (Mendelian segregation) if they were hybrids (produced by fertilization of eggs of the melanic mate by sperm of the albino), and that only melanic F_2s were produced if there had been no cross-fertilization of the F_1 eggs.

Each crossing experiment involved a melanic (M) *B. occidentalis* and an albino (A) *B. tenagophila*, as follows (Fig. 13):

1. M from Guaíra x A from Asunción: 20 couples;
2. M from Guaíra x A from Joinville: 20 couples;
3. M from Guaíra x A from Taubaté: 20 couples;
4. M from Bela Vista x A from Asunción: 10 couples;
5. M from Campo Grande x A from Asunción: 10 couples;

6. M from Miranda x A from Asunción: 5 couples;
7. M from Cuiabá x A from Asunción: 5 couples;
8. M from Cáceres x A from Asunción: 5 couples;
9. M from Pontes e Lacerda x A from Asunción: 10 couples;
10. M from Pontes e Lacerda x A from Joinville: 10 couples;
11. M from Pontes e Lacerda x A from Taubaté: 10 couples.

When each crossing experiment was over, each not cross-fertilized albino was mated to a homozygous melanic *B. tenagophila* from its own population (Taubaté, Joinville or Asunción) to control its capacity to be cross-fertilized. (By the way, previous experiments had shown that the populations of *B. tenagophila* from the three mentioned localities are freely interfertile.) As to the melanic *B. occidentalis* which failed to fertilize its albino mate, it was dissected to control the soundness of its genital system and the possibility of any hindrance to sperm flow.

Additional crossings were attempted between M from Guaíra, Pontes e Lacerda and Cuiabá, and A *B. glabrata* from Santa Luzia, Minas Gerais state (10 couples for each combination).

Owing to the likeness of *B. occidentalis* to *B. tenagophila,* their genital systems are shown for comparison in Figs. 3, 4, 5-8 and 9-12. I collected the specimen of Fig. 4 in June 1973 at Yacarey (a name now being changed to Curuzú), on the road from Corrientes to Posadas (Ruta National No. 12), Argentina. The type locality of *B. tenagophila,* as mentioned in Orbigny's original description (1835 :26), is the Argentine province of Corrientes, more precisely the "canton de las Ensenadas" (Orbigny, 1837 :347), which now corresponds to the "Departamento de San Cosme". Since Yacarey is only 15 km distant from the western limit of the "canton de las Ensenadas", it is situated practically in the area mentioned by Orbigny.

RESULTS

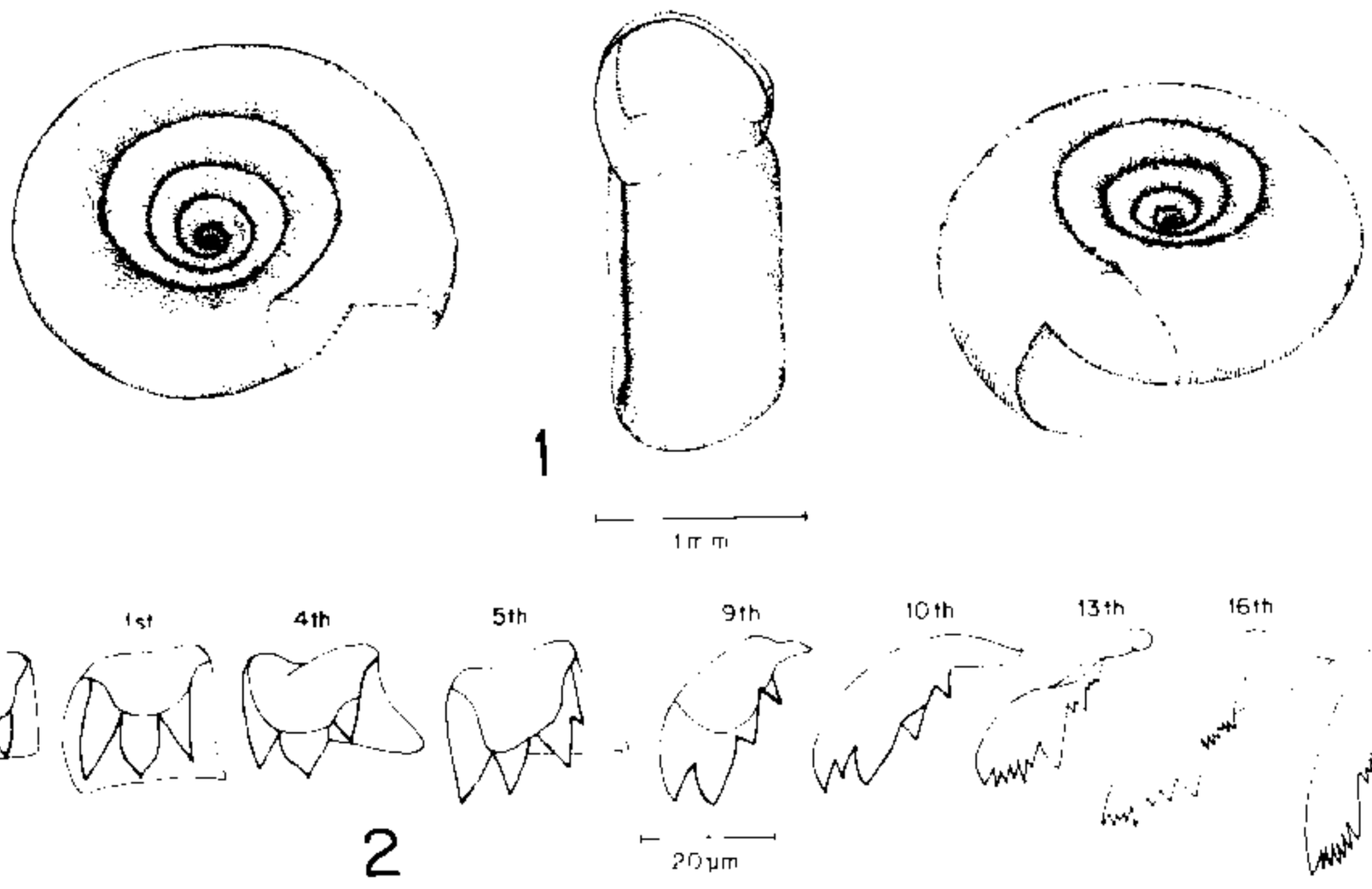
Morphology

The largest specimens, measuring 21 mm in diameter by 8 mm in width, and with 6.5 to 7 whorls, were found at Campo Grande and Guaíra.

The shell (Fig. 1) is indistinguishable from that of *B. tenagophila* as described by Paraense & Deslandes (1955). The whorls increase slowly in diameter. Both sides are moderately concave, with all whorls plainly visible and more or less sharply carinate. The carina is more evident on the outer whorl, especially on the left side; on the inner whorls it often coincides with the suture, so as to become inapparent. In these instances the suture is level with the lateral surface of the shell. The periphery is rounded and somewhat shifted to the right. The aperture is heart-shaped or deltoid, usually transverse, and has an angular left lip; it is directed forward, and its ventrodorsal axis is frequently bent to the right.

A remarkable external characteristic of *B. occidentalis,* in common with *B. tenagophila,* is the width of its shell, proportionally wider than in the other South American biomphalarias. Owing to natural variation, however, narrower shells are found in all samples, more frequently in some populations, as those from Sena Madureira, Miranda and Pontes e Lacerda.

The cephalopedal mass and the pallial organs have no features of special interest. The ventral surface of the renal tube is even and smooth, showing no renal ridge.



Biomphalaria occidentalis sp. n. – Fig. 1: shell of largest specimen from Campo Grande, 21mm in diameter. Fig. 2: radula of largest specimen from Guaíra, 21mm in diameter (C = central tooth; remaining teeth numbered according to position on horizontal row).

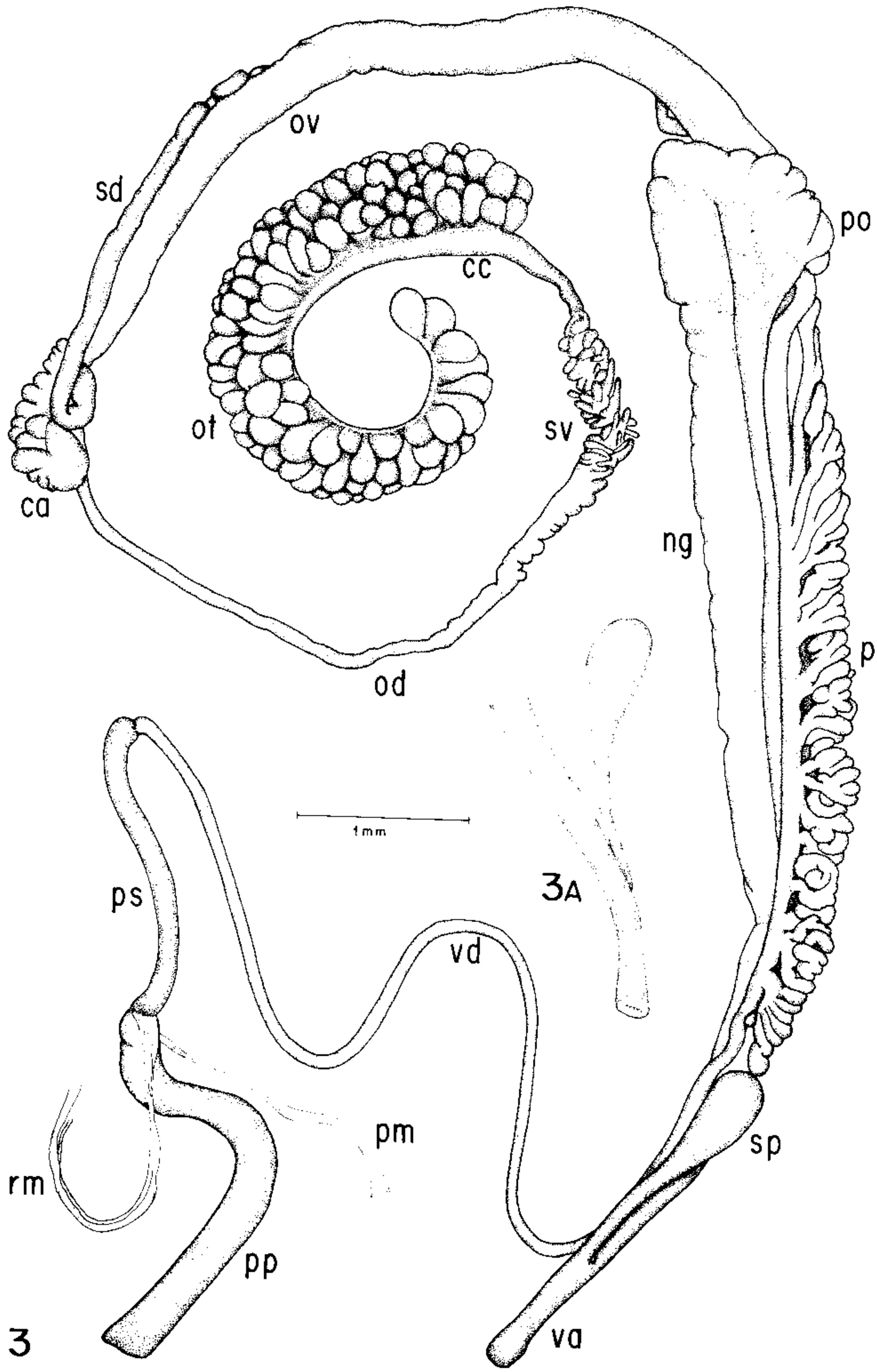
The radula shows no significant differences from that of other biomphalarias. The largest dissected specimen, 21mm in shell diameter, had the radular formula 26-1-26, with a bicuspid central tooth, 6 laterals, 4 intermediates and 16 marginals (Fig. 2), and 129 horizontal rows of teeth. Another specimen showed radular anomalies: central tooth unicuspid (right cuspid lacking), first left lateral with bifurcate entocone, and third left lateral acuspidate.

The ovotestis (Fig. 3, ot) has over 100 diverticula, mostly simple or bifurcate, but a number of them are divided into more branches. The ovotestis of one specimen about 20mm in shell diameter was thoroughly dissected, showing 140 diverticula, of which 64 were unbranched, 54 bifurcate, 14 trifurcate, 7 quadrifid and 1 quinquifid. A specimen of *B. tenagophila* about the same size, dissected for comparison, showed 139 diverticula: 71 unbranched, 54 bifurcate, 9 trifurcate and 5 quadrifid.

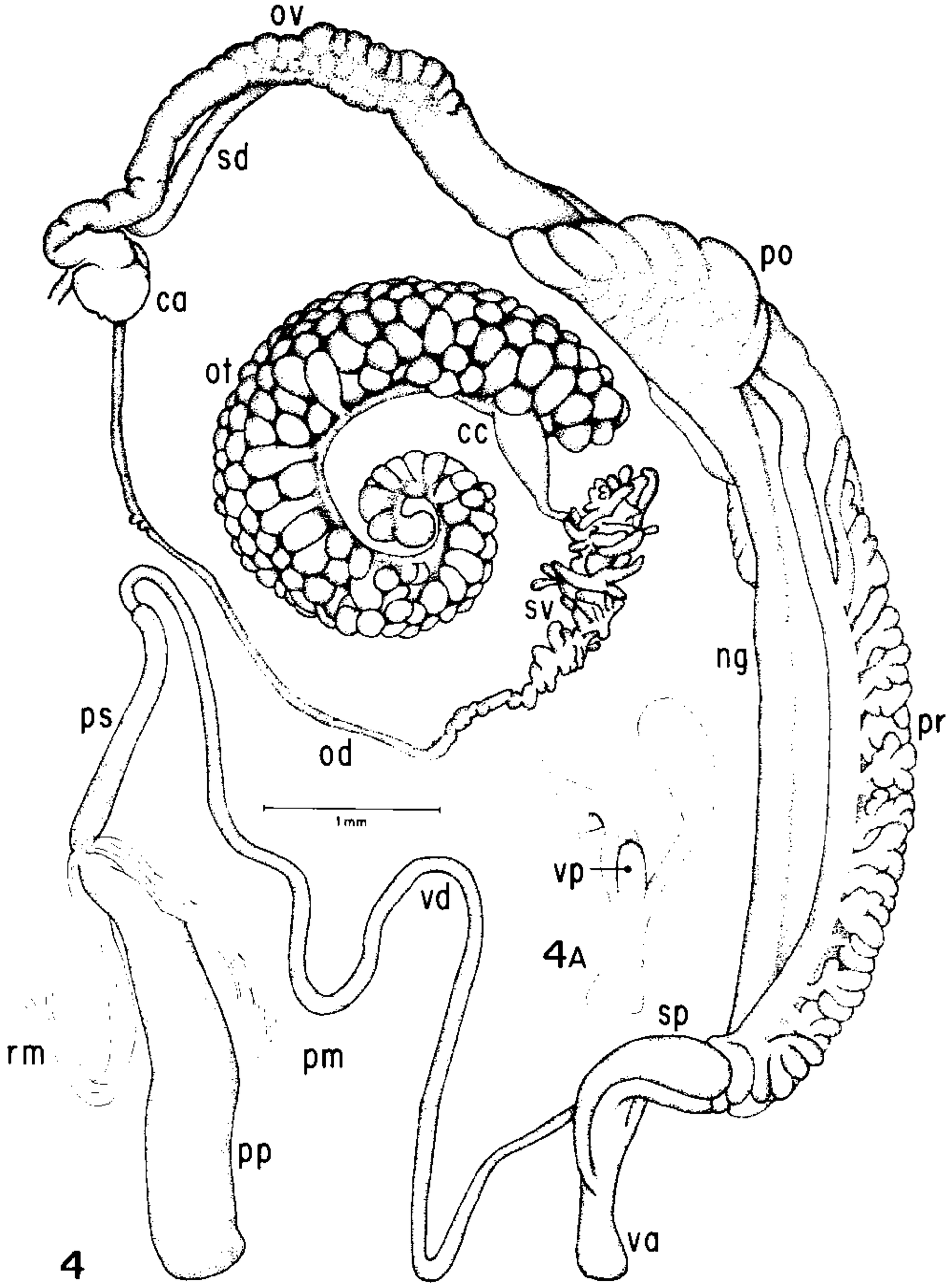
The seminal vesicle (Fig. 3, sv) is beset with parietal diverticula which vary in shape from knob-like to finger-like.

The oviduct, the pouch of the oviduct and the nidamental gland (Fig. 3, ov, po, ng) have no special characteristics. The vagina (Figs. 3, va, and 3A) is long and tubular, showing a smooth surface in the great majority of specimens, with no trace of the vaginal pouch characteristic of *B. tenagophila* (Figs. 4A, vp, and 10-12). In some individuals there is a slight projection of the vaginal wall, as shown in Figs. 6-8, which may be mistaken for a pouch by a less acquainted observer. However, it is not clearly demarcated from the surrounding surface as in the case of a true pouch. The spermatheca (Figs. 3, sp, and 6-8) has a usually club-shaped body and a slender duct about as long as the body. The shape of the spermathecal body, however, may vary from ovoid to rounded, according to its state of repletion.

The spermiduct (Fig. 3, sd) runs adherent to the oviduct, as usual. After traversing the furrow of the pouch of the oviduct, it puts forth a series of 12 to 25 prostatic diverticula, most of which are moderately arborescent (Fig. 3, pr). According to Paraense

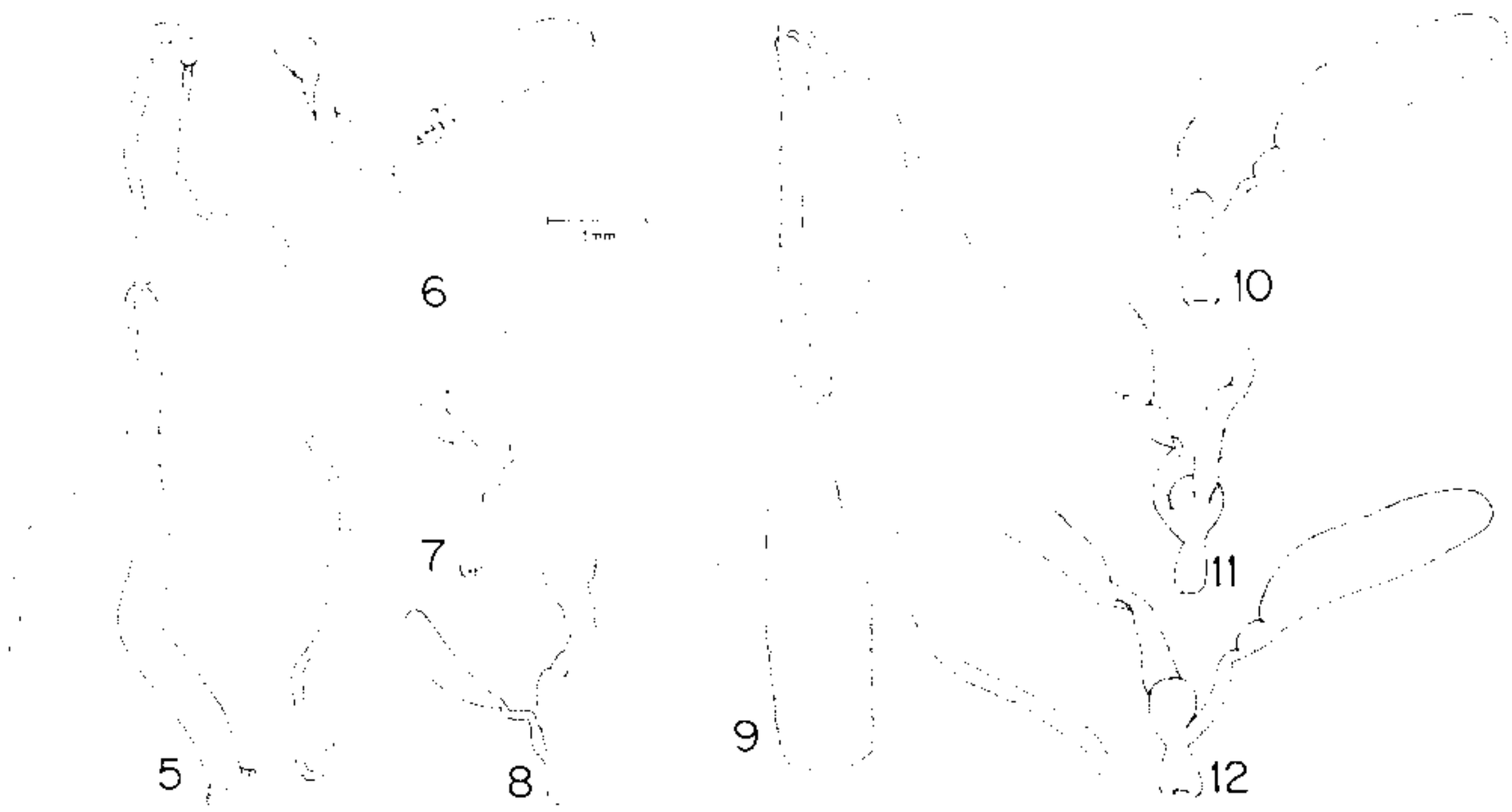


Biomphalaria occidentalis sp. n. – Fig. 3: genital system, specimen from Campo Grande, compare with Fig. 4 (ca = carrefour, cc = collecting canal of ovotestis, ng = nidamental gland, od = ovispermiduct, ot = ovotestis, ov = oviduct, pm = protractor muscle of penial complex, po = pouch of oviduct, pp = prepuce, pr = prostate, ps = penial sheath, rm = retractor muscle of penial complex, sd = spermiduct, sp = spermatheca, sv = seminal vesicle, va = vagina, vd = vas deferens). Fig. 3A: vaginal complex, ventral view.



Biomphalaria tenagophila – Fig. 4: genital system, specimen from Yacarey, Argentina (abbreviations as in Fig. 3). Fig. 4A, vaginal complex, ventral view (vp = vaginal pouch).

(1975), the number of prostatic diverticula in *B. tenagophila* ranges from 12 to 26, but subsequent observations showed a minimum of 10 and a maximum of 27, in specimens from Jacareí (São Paulo state) and Paso de los Libres (Corrientes province, Argentina), respectively. The foremost prostatic diverticulum, in *B. occidentalis* as well as in *B. tenagophila,* either merely contacts (Fig. 3, pr) or surrounds the top of the spermatheca, in this case becoming inserted between the spermatheca and the uterine wall. The vas deferens (Fig. 3, vd) has its widest portion narrower than the penial sheath (Fig. 3, ps). The latter, uniformly cylindrical in shape, is slender and shorter than the prepuce in the majority of cases, the ratio between the lengths of the two organs varying from 0.40 to 1.00 (in *B. tenagophila* it is about 0.50 to 1.50). The prepuce (Fig. 3, pp) is wider than the penial complex and keeps about the same diameter throughout its length, whereas in *B. tenagophila* it is much wider than the penial sheath and tends to expand distalward (Fig. 4, pp). The main retractor and protractor muscles of the penial complex are more slender than in *B. tenagophila* (compare Figs. 3 and 4, rm, pm).



Biomphalaria occidentalis – Fig. 5: penial complex, specimen from Guaíra. Fig. 6: vaginal complex, ventral view, same specimen of Fig. 5. Fig. 7: vaginal complex, ventral view, specimen of Várzea Grande. Fig. 8: vaginal complex, dorsal view, same specimen of Fig. 7.

Biomphalaria tenagophila from Macaé – Fig. 9: penial complex. Figs. 10-12: vaginal complex, dorsoventral (10), dorsal (11) and ventral (12) views.

Crossing Experiments

No cross-fertilization occurred in any of the matings between the melanic specimens from Guaíra, Bela Vista, Campo Grande, Miranda, Cuiabá, Cáceres and Pontes e Lacerda, and the albino *B. tenagophila* from Asunción, Joinville or Taubaté. Not only did the albinos produce exclusively albino offspring, but also the F_2 generation from the melanic mates consisted of exclusively melanic specimens, showing that both mates were reproducing by self-fertilization. On the other hand, all albino mates were readily fertilized by melanic specimens from their own populations to which they were paired after each experiment; and no abnormalities were found in the genital system of the melanic *B. occidentalis* previously mated to them.

Crossing experiments between *B. occidentalis* from Guaíra and Pontes e Lacerda, and *B. glabrata* from Santa Luzia, also gave negative results.

DISCUSSION

As the shell of *B. occidentalis* is indistinguishable from that of *B. tenagophila*, the only way of separating the two species by morphological characteristics is through examination of the reproductive system, where the only significant differences are to be found in the vaginal wall and in the penial complex (penial sheath and prepuce). Such differences were pointed out above (see "Morphology").

The absolute reproductive isolation between *B. occidentalis* and *B. tenagophila* affords conclusive biological evidence of specific distinctness.

As shown by Paraense (1956, 1959), in *B. glabrata* the degree of fertility between individuals from different populations decreases as the geographical distance between the populations increases. In the mentioned experiments it was observed that a distance of about 2000 km is compatible with interfertility, merely reducing to some extent the number of hybrids produced. For greater distances the reduction is more and more drastic. Moreover, such reduction in hybrid production is brought about by a mechanism that, in *B. glabrata*, hampers fertilization of eggs of southern individuals by sperm of northern ones and is less effective, even apparently ineffective, in the opposite direction. In the present work, as albino *B. tenagophila* were only available from Asunción, Joinville and Taubaté, melanic *B. occidentalis* from Guaíra were used in proportionally greater number owing to their closer proximity to the other three localities, but additional tests involving melanic *B. occidentalis* from more distant places were also performed (Fig. 13).

It is commonly observed that two conspecific planorbids reared in isolation and placed together after reaching sexual maturity will soon meet and start copulation, usually in 1 hr or less. Although in the control tests involving albino and melanic *B. tenagophila* from the same population, referred to under "Material and Methods", the mates consistently behaved in the mentioned way, no attempts at copulation between *B. occidentalis* and *B. tenagophila* were observed; one of the mates mounted upon the other's shell simply to browse on the layer of microorganisms which grow on isolated specimens. It is reasonable, therefore, to presume that some kind of ethological isolating mechanism operates in the case of *B. occidentalis* with *B. tenagophila*.

As mentioned under "Material and Methods", before fixation the snails were relaxed in a 0.1% solution of nembutal. Till then a 0.2% solution was currently used, but it was observed that *B. occidentalis*, unlike *B. tenagophila* and all the other Brazilian biomphalarias dealt with previously, retracted into the shell when placed in the solution. Halving the concentration, *B. occidentalis* relaxed satisfactorily as well as other species tested (*B. tenagophila, B. glabrata, B. straminea, B. peregrina* and *Helisoma duryi*). This observation points to an interspecific difference in reaction to the drug.

In addition to *B. tenagophila*, shells of South American planorbids with lateral carination have been described under the names *Planorbis ferrugineus* Orbigny, 1835; *P. andecolus* Orbigny, 1835; *P. bahiensis* Dunker, 1850; *P. biangulatus* Sowerby, 1877; *P. clevei* "Jousseaume" Cousin, 1887; *P. paysanduensis* Marshall, 1930; and *Australorbis amphiglyptus* Pilsbry, 1951.

Setting aside *P. andecolus*, which is a good species (see Paraense & Deslandes, 1957), the nomenclatural status of the remaining ones is as follows:

a. *Planorbis ferrugineus* Orbigny (not *P. ferrugineus* "Spix" Wagner, 1827) and *P. bahiensis* Dunker, in so far as they refer to specimens from Rio de Janeiro city, are synonyms of *B. tenagophila* (discussion in Paraense, 1961b);

b. *P. biangulatus*, said by Sowerby (1877) to be from Brazil, was considered a synonym of *Planorbis nigricans* (= *Biomphalaria tenagophila*) by Lutz (1918); in the

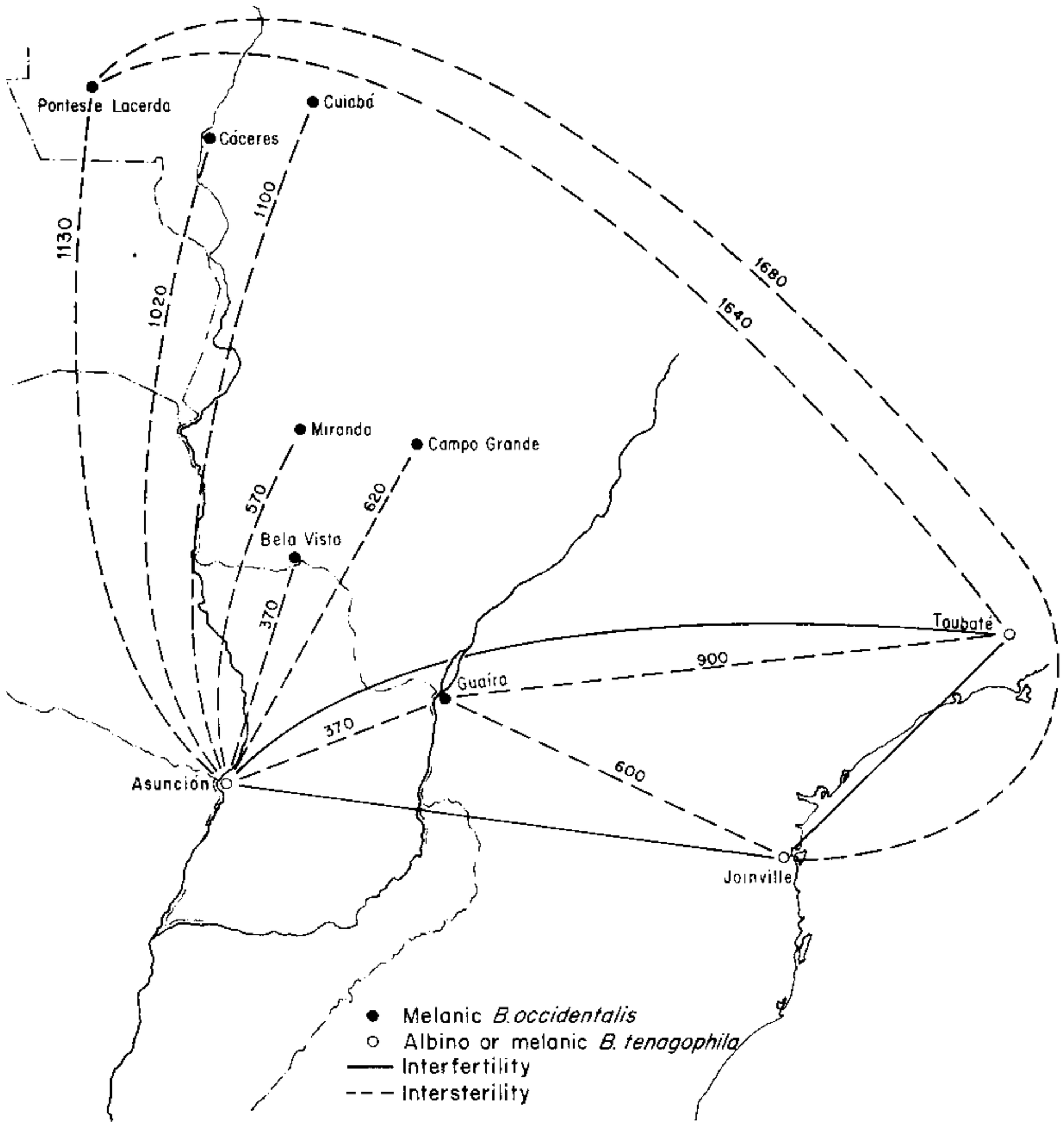


Fig. 13: Localities from which specimens of *Biomphalaria occidentalis* and *B. tenagophila* were used in crossing experiments. Distances between localities in km.

absence of a more restricted locality, it is impossible to define this nominal species on the basis of the shell characters alone;

c. *P. paysanduensis* Marshall from Paysandú (Uruguay), many specimens of which were collected by myself at that locality in March and October, 1965, is identical in shell and anatomical characters to *B. tenagophila*, and freely hybridizes with South Brazilian representatives of this species;

d. *A. amphiglyptus* Pilsbry, as shown by Paraense (1961b), is another synonym of *B. tenagophila;*

e. *P. clevei* is one of the nearly 300 species of molluscs from Ecuador studied by Cousin (1887 : 263), who spent many years in that country. The shell is finely described and figured, and its origin is said to be "Équateur". During a collecting trip to Ecuador, in

April 1965, I had the opportunity of examining, at the Jijón y Caamaño Library, Quito, a reprint of Cousin's paper annotated by himself, on which he added that *P. clevei* was from "Guayaquil, Équateur. Yaguachi. Mapasinga". My search in this area coincided with heavy rains, so that only four empty shells answering perfectly to Cousin's description and figures were found in a flooded lake at Pascuales. Therefore it is impossible, for the present, to discuss the relationship between *B. occidentalis* and *P. clevei.*

Barbosa et al (1963) described a planorbid with carinate shell from Los Ríos, Ecuador, which they identified with *Planorbis sericeus*, a name given by Dunker (1848) to a shell of unknown origin ("Patria ignota") of Cuming's collection in the British Museum (Natural History). Its resemblance to *B. tenagophila* was remarked by Dunker: "Testa *Pl. tenagophilo,* Orb., affinis, sed magis involuta et regularis". Although the shape of the shell and the smoothness of the vaginal wall approach *B. occidentalis* and the snail studied by Barbosa et al (1963), the prepuce of the latter is much wider, in proportion to the penial sheath, than it would be expected of *B. occidentalis.*

The occurrence in South America of so many nominal species with similar shells emphasizes the unreliability of the shell characters alone in species discrimination (Paraense, 1961a).

GEOGRAPHICAL DISTRIBUTION

Fig. 14 shows the distribution of *B. occidentalis* and *B. tenagophila*, according to the records of this laboratory. Owing to the likeness of their shells, only dissected specimens were considered. Locality 113 (Tefé) is represented by empty shells, tentatively identified with *B. occidentalis* because of its great distance of the range of *B. tenagophila.* In locality 137 (Asunción) the two species were found in separate biotopes: a single specimen of *B. occidentalis*, 12mm in shell diameter, was collected by myself, in March 1965, from Laguna Colorada at Paso Bareiro, flooded by heavy rainstorms in the preceding days; and a batch of *B. tenagophila* from Bañado y Arroyo Ferreira, collected by Lic. María Mercedes Culzoni, Universidad Nacional de Asunción, and including a few albino specimens, was sent to this laboratory for identification in 1978.

RESUMO

É descrita uma nova espécie de molusco planorbídeo sul-americano, *Biomphalaria occidentalis,* indistinguível da *B. tenagophila* (Orbigny, 1835) pelos caracteres da concha e da maioria dos órgãos do sistema genital. Na *B. tenagophila* existe uma bolsa na parede ventral da vagina (Fig. 4A, vp), ausente na *B. occidentalis* (Fig. 3A), e o prepúcio é muito mais grosso que a bainha do pênis, aumentando de largura na direção distal (Fig. 4, ps, pp), ao passo que na *B. occidentalis* o prepúcio é mais grosso que a bainha do pênis porém seu diâmetro pouco se altera em toda sua extensão (Fig. 3, ps, pp).

As duas espécies são biologicamente separadas por isolamento reprodutivo absoluto.

A Fig. 14 mostra a distribuição geográfica da *B. occidentalis*, que até agora foi encontrada nos Estados brasileiros do Acre, Amazonas (?), Mato Grosso, Mato Grosso do Sul e Paraná, e no Paraguai. Sua localidade-tipo é Campo Grande, Estado de Mato Grosso do Sul, onde foi coletada em vários biótopos relacionados a afluentes do rio Aquidauana, principalmente nos córregos Prosa e Ceroula.

Foram depositados exemplares nas seguintes coleções malacológicas: Instituto Oswaldo Cruz, Rio de Janeiro; Academy of Natural Sciences, Philadelphia; Museum of Zoology, University of Michigan; British Museum (Natural History).

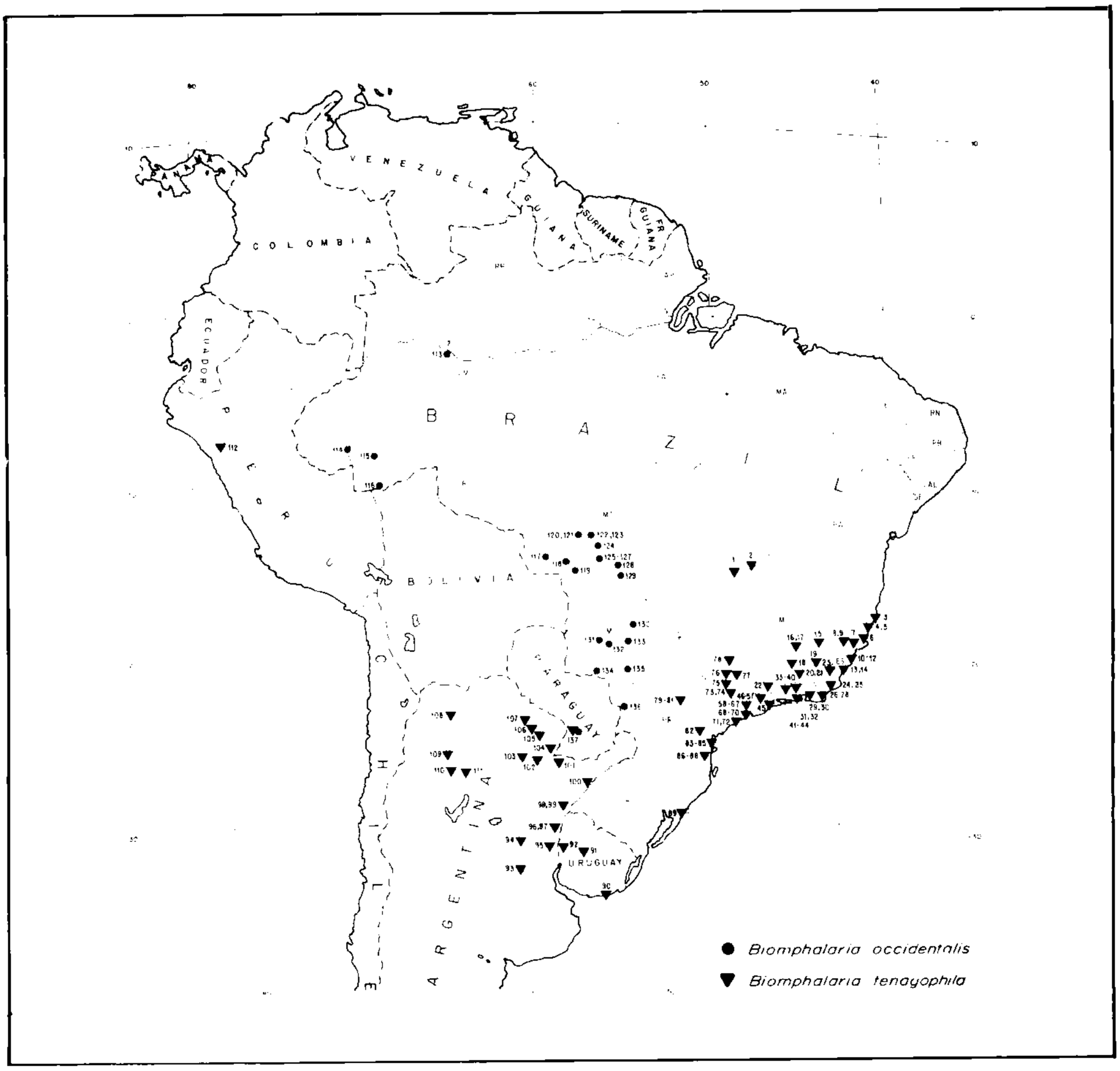


Fig. 14: Distribution of populations of *Biomphalaria tenagophila* and *B. occidentalis* examined in this study; discrimination based on anatomical characteristics.

B. tenagophila – 1: Brasília, 2: Formosa, 3: Caravelas, 4: Conceição da Barra, 5: São Mateus, 6: Linhares, 7: Colatina, 8: Aimorés, 9: Pancas, 10: Vitória, 11: Vila Velha, 12: Cariacica, 13: Castelo, 14: Marataízes, 15: Nova Era, 16: Belo Horizonte, 17: Betim, 18: Barbacena, 19: Ubá, 20: Rio Novo, 21: Juiz de Fora, 22: Itajubá, 23: Itaperuna, 24: São Joaquim, 25: Campos, 26: Macaé, 27: São Pedro da Aldeia, 28: Cabo Frio, 29: São Gonçalo, 30: Niterói, 31: Duque de Caxias, 32: Rio de Janeiro, 33: Cantagalo, 34: Cordeiro, 35: Sapucaia, 36: Três Rios, 37: Paraíba do Sul, 38: Itaipava, 39: Petrópolis, 40: Teresópolis, 41: Vassouras, 42: Barra do Piraí, 43: Paracambi, 44: Piraí, 45: São Luís do Paraitinga, 46: Cruzeiro, 47: Cachoeira Paulista, 48: Piquete, 49: Lorena, 50: Guaratinguetá, 51: Aparecida, 52: Roseira, 53: Pindamonhangaba, 54: Tremembé, 55: Taubaté, 56: Caçapava, 57: Jambeiro, 58: São José dos Campos, 59: Jacareí, 60: Santa Isabel, 61: Guarulhos, 62: Suzano, 63: São Caetano do Sul, 64: São Paulo, 65: Itapecerica da Serra, 66: Santo André, 67: São Bernardo do Campo, 68: Cubatão, 69: São Vicente, 70: Santos, 71: Ana Dias, 72: Itariri, 73: Porto Feliz, 74: Campinas, 75: Piracicaba, 76: São Carlos, 77: Pirassununga, 78: Ribeirão Preto, 79: Uraí, 80: Londrina, 81: Assaí, 82: Curitiba, 83: Morretes, 84: Antonina, 85: Paranaguá, 86: Joinville, 87: Itajaí, 88: Blumenau, 89: Tramandaí, 90: Maldonado, 91: Arroyo Salsipuedes; 92: Paysandú, 93: Rosario, 94: Santa Fe, 95: San Salvador, 96: Concordia, 97: Federación, 98: Curuzú-Cuatiá, 99: Paso de los Libres, 100: Santo Tomé, 101: Yacarey, 102: Lapachito, 103: Presidente Roque Saenz Peña, 104: Tres Mojones, 105: Los Matacos, 106: Apeadero Bruchard, 107: Las Lomitas, 108: San Salvador de Jujuy, 109: Leocadio Paz, 110: Aquilares, 111: Termas de Río Hondo, 112: Cajabamba, 137: Asunción.

B. occidentalis – 113: Tefé (only shells available), 114: Tarauacá, 115: Sena Madureira, 116: Brasiléia, 117: Pontes e Lacerda, 118: Cabaçal, 119: Cáceres, 120: Nortelândia, 121: Arenápolis, 122: Diamantino, 123: Alto Paraguai, 124: Rosário Oeste, 125: Cuiabá, 126: Várzea Grande, 127: Nossa Senhora do Livramento, 128: Santa Elvira, 129: Rondonópolis, 130: Camapuã, 131: Miranda, 132: Aquidauana, 133: Campo Grande, 134: Bela Vista, 135: Rio Brilhante, 136: Guaíra, 137: Asunción.

ACKNOWLEDGMENTS

I am indebted to Dr. C. Edgardo Borda, Director, Centro Nacional de Parasitología, Universidad Nacional del Nordeste, Argentina, for the information about the limits of the former "canton de las Ensenadas"; to Dr. José Taquarussu Fiuza Lima, Superintendent of SUCAM (Superintendency of Public Health Campaigns, Ministry of Health), Dr. Solon de Camargo, former Director, Schistosomiasis Division of SUCAM, to the Regional Directors of SUCAM, Drs. Roraima Moreira da Rocha (Acre), José Joaquim Fonseca Sandoval (Amazonas), Judá Dantas Vanderlei (Mato Grosso), Edir Daubian (Mato Grosso do Sul) and Alcides Klug (Paraná), for innumerable facilities for work in the areas of their jurisdiction; to the Pan American Health Organization, for sponsoring collecting trips to Argentina, Uruguay, Paraguay and Ecuador; and to the National Research Council (CNPq) for sponsoring the work within this country.

REFERENCES

BARBOSA, F.S.; BARBOSA, I. & CARNEIRO, E., 1963. Description of *Australorbis sericeus* (Dunker), a possible intermediate host of *Schistosoma mansoni* in Ecuador. *Ann. Trop. Med. Parasitol. 57* (1) :52-58.

COUSIN, A., 1887. Faune malacologique de la République de l'Équateur. *Bull. Soc. Zool. France 12* :187-287.

DUNKER, W., 1848. Diagnoses specierum novarum generis *Planorbis* collectionis Cumingianae. *Proc. Zool. Soc. London 16* :40-43.

DUNKER, W., 1850. *Planorbis* (in part). In MARTINI & CHEMNITZ: Systematisches Conchylien-Cabinet, Ed. 2, Vol. 1, pt. 17. Bauer & Raspe, Nürnberg.

LUTZ, A., 1918. Caramujos de água doce do gênero *Planorbis*, observados no Brazil. *Mem. Inst. Oswaldo Cruz 10* (1) :65-82 (English version :45-61).

MARSHALL, W.B., 1930. New land and fresh-water mollusks from South America. *Proc. U.S. Nat. Mus.* 77, Art. 2, No. 2825 :1-7.

ORBIGNY, A., 1835. Synopsis terrestrium et fluviatilium molluscorum, in suo per American meridionalem itinere collectorum. *Mag. Zool.* 5, Classe 5 (61-62) :1-44.

ORBIGNY, A., 1837. Voyage dans l'Amérique méridionale, vol. 5, 3^{e} partie: Mollusques. P. Bertrand, Paris.

PARAENSE, W.L., 1955. Self – and cross – fertilization in *Australorbis glabratus. Mem. Inst. Oswaldo Cruz 54* (2/4) :285-291.

PARAENSE, W.L., 1956. A genetic approach to the systematics of planorbid molluscs. *Evolution 10* (4) :403-407.

PARAENSE, W.L., 1959. One-sided reproductive isolation between grographically remote populations of a planorbid snail. *Amer. Nat. 93* (869) :93-101.

PARAENSE, W.L., 1961a. Shell versus anatomy in planorbid systematics. I. *Australorbis glabratus. Rev. Brasil. Biol. 21* (2) :163-170.

PARAENSE, W.L., 1961b. The nomenclature of Brazilian planorbids. II. *Australorbis tenagophilus* (Orbigny, 1835). *Rev. Brasil. Biol. 21* (4) :343-349.

PARAENSE, W.L., 1975. Estado atual da sistemática dos planorbídeos brasileiros. *Arq. Mus. Nac., Rio de Janeiro, 55* :105-128.

PARAENSE, W.L. & DESLANDES, N., 1955. Observations on the morphology of *Australorbis nigricans*. *Mem. Inst. Oswaldo Cruz 53* (1) :121-134.

PARAENSE, W.L. & DESLANDES, N., 1957. A redescription of *Taphius andecolus* (Orbigny, 1835). *Rev. Brasil. Biol. 17* (2) :235-243.

PILSBRY, H.A., 1951. Notes on some Brazilian Planorbidae. *Nautilus 65* (1) :3-6.

SOWERBY, G.B., 1877. Monograph of the genus *Planorbis*. In L. A. REEVE: Conchologia Iconica, vol. 20. L. Reeve & Co., London.

Note added in proof. – I extended the collections to the western area of São Paulo state near Mato Grosso do Sul, identifying *B. occidentalis* at Presidente Epitácio, Mirante do Paranapanema, Dracena, Presidente Prudente and Valparaíso, and *B. tenagophila* at Araçatuba. I am grateful to Dr. George Ishihata, Superintendent of SUCEN (Superintendency for Control of Endemic Diseases, Department of Health, São Paulo state), and to other members of its staff, Drs. Jorge Faria Vaz, Head of the Malacological Laboratory, Rogerio Christensen, Regional Director at Presidente Prudente, and Luiz Takaku, Regional Director at Araçatuba, who provided facilities for field work in the area.