Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 83(1): 1-12, jan./mar. 1988

BIOMPHALARIA KUHNIANA (CLESSIN, 1883), PLANORBID MOLLUSC FROM SOUTH AMERICA

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The validity of Biomphalaria kuhniana *(Clessin, 1883) is confirmed through morphological study of specimens from Surinam (type locality) and the area of Tucurui (Tocantins river, state of Pará, Brazil) in comparison with* B. straminea *(Dunker, 1848), and through crossing experiments which revealed complete reproductive isolation between the two species. The full-grown shell of* kuhniana *is smaller (about 7.5 mm) than that of* straminea *(11 mm to 16.5 mm). Anatomically they differ in the degree of corrugation of the vaginal wall (little developed in* kuhniana, *conspicuous in* straminea*), number and shape of prostatic diverticula (*kuhniana *4 to 9, shorter and less branched;* straminea *9 to 18, longer and more branched), number of muscle layers at the middle of the penis (two in* kuhniana, *three in* straminea*), distal segment of the spermiduct usually straight or slightly wavy in* kuhniana, *more or less curly in* straminea.

Differences between B. kuhniana *and* B. intermedia *(Paraense & Deslandes, 1962) are less marked. The latter has a shell up to about 12 mm in diameter, 7 to 15 prostatic diverticula, two muscle layers at the middle of the penis, and a vaginal wall with a combination of a more or less developed corrugation (or sometimes a mere swelling) on the left of the spermathecal duct and a rudimentary pouch on the right of the duct.*

A Biomphalaria straminea *complex is proposed to include that species as well as* B. kuhniana *and* B. intermedia.

Key words: Mollusca – Planorbidae – *Biomphalaria kuhniana* – *Biomphalaria straminea* – *Biomphalaria intermedia* – Sibling species – Taxonomy

The species studied in this paper was described by Clessin (1883: 108-109, Pl. 11, Fig. 12) as follows:

> 72. *Planorbis Kühnerianus* Dunker.
> Taf. 11. Fig. 12.
> T. parvula, utrinque concava centro profunde immerso, tenuis, diaphana, nitidula, leviviter striatula, pallide cornea; anfr. 4, rotundati, celeriter regulariterque accrescentes; ultimus penultimo latior; sutura supra maxime, infra sat profunda; apertura obliqua, late-lunata; peristoma acutum, marginibus callo tenui conjunctis.
> Diam. 6,5, alt. 2 Mm.
> *Planorbis Kühnerianus* in coll.
> Gehäuse klein, beiderseits concav mit tief eingesenktem Mittelpunkte, dünnschalig, durchscheinend, glänzend, fein gestreift, gelblich-hornfarben; Umgänge 4, rundlich, rasch und regelmässig zunehmend, der letzte doppelt so breit als der vorletzte; Naht oben sehr, unten mässig tief; Mündung schief, breit mondförmig: Mundsaum scharf, Ränder durch eine dünne Schwiele verbunden.
> Vaterland: Surinam (Coll. Dkr.).

The species name *Kühnerianus* was emended to *Kühnianus* by Clessin (1886 – errata: 411, summary of plates: 413, index: 429).

This planorbid has been mentioned by few workers. Vernhout (1914: 23) referred to a single shell from the environs of Paramaribo represented in the collection of the Leyden Museum. H. B. Baker (1930: 46-47) described the jaw and radula of specimens from Bejuma and Boquerón (Venezuela), identified as *Planorbula (Tropicorbis) kuhniana*, which showed no special features; some shells from Bejuma appear in F. C. Baker's (1945, Pl. 132, Figs. 6-9) monograph. Floch & Lajudie (1945) and Floch & Fauran (1954a, b) recorded it in Cayenne and surroundings (French Guiana), their specimens having been identified as *Tropicorbis kuhnianus* by J. Bequaert (Museum of Comparative Zoology, Harvard College) and H. A. Rehder (U. S. National Museum). Ranson

Received June 6, 1987.
Accepted June 30, 1987.

(1955) figured the prostate of a specimen of unstated origin to justify its placing in the genus *Biomphalaria.* In Harry's (1964) catalogue *Planorbis kuhniana* was placed incertae sedis among the Neotropical planorbids.

In 1959 I took an interest in identifying some populations of a small planorbid from the northernmost region of Brazil, the shell of which scarcely reached 7 mm in diameter. Anatomically they resembled *Biomphalaria straminea* but had fewer prostatic diverticula and a less developed corrugation on the vaginal wall. Examining the literature on the planorbids of the area, the possibility was considered of the species under investigation being *Planorbis kuhnianus.* Consequently, I collected in Paramaribo a number of specimens answering perfectly to Clessin's description of *Planorbis kuhnianus.* Anatomically it resembled *Biomphalaria straminea* but had fewer prostatic diverticula and a less developed corrugation on the vaginal wall. Finding no grounds to separate the two taxa, I treated them as synonyms (Paraense, 1963: 2). A small sample from Saramacca, sent to this laboratory for identification, in 1964, by Dr. Deryck W. Heinemann, Department of Public Health of Surinam, proved indistinguishable from the Paramaribo specimens. More recently, examining a small *Biomphalaria* from the area of the Tucuruí dam, on the Tocantins river (Pará state), at Tucuruí (3°43'S, 49°42'W), Jatobal (4°33'S, 49°33'W) and Itupiranga (5°09'S, 49°21'W), I noticed a great similarity between it and the planorbid from Surinam. Crossing experiments between specimens from Tucuruí and *B. straminea* revealed complete reproductive isolation.

This paper is concerned with the morphology (shell and anatomy) of the snails from Surinam, the type locality of *P. kuhnianus,* and from the area of Tucuruí, and with a comparison between them and a population of *B. straminea,* including the results of crossing experiments.

MATERIAL AND METHODS

Fifteen snails were collected in Paramaribo, on 23 Oct. 1959, from a swampy area connected with a slow-flowing creek, where they occurred in syntopy with *Biomphalaria glabrata* and *Drepanotrema anatinum.* No additional specimens were found during a two days' search in numerous bodies of water over the town and surroundings. Each specimen was killed by gradual immersion in water heated at 70°C, with the aperture upwards, so carefully as to minimize its retracting back to the shell. After 15 sec the snail was completely plunged for 20 additional sec and then transferred to water at room temperature. The animal (under water) was drawn from the shell with a small forceps applied to the neck, and fixed in Railliet-Henry's fluid (distilled water 930 ml, sodium chloride 6 g, formalin 50 ml, glacial acetic acid 20 ml). Ten larger specimens were dissected for anatomic study. One specimen was embedded in paraffin, sectioned serially and stained with hematoxylin-eosin for microanatomic observation. The radulae were separated from the buccal mass by digestion in a vial with 10% NaOH left overnight in the incubator at 56°C. They were then rinsed in tap water and mounted in a drop of glycerin on a microscopic slide, with the dorsal (toothed) surface upwards as in the living animal.

The sample from Saramacca was collected from a borrow pit at Damboentong in March 1964. Five specimens were treated as described above for anatomic dissection.

The specimens from the area of Tucuruí were collected from ponds and swamps at the above-mentioned localities, from 12 Oct. to 2 Nov. 1983, usually together with *Drepanotrema anatinum* and *D. lucidum.* Forty specimens from Tucuruí were relaxed, before fixation, in a 0.05% solution of nembutal for about 6 hr, then immersed, without special care (in groups, in a small strainer), for 40 sec, in hot water at 70°C, and thereafter treated as described above.

Forty *B. straminea* from Tangará, 6.5 to 8 mm in shell diameter, were dissected for comparison with the Tucuruí specimens.

Crossing experiments were performed between 40 pairs of snails kept in isolation since hatching and laying eggs regularly, using albinism as genetic marker (Paraense, 1955). A specimen from Tucuruí (of which only melanic individuals have been found so far) was put in a small aquarium with an albino *B. straminea* of about the same size from Tangará (6°12'S, 35°49'W), Rio Grande do Norte state, and left together for 10 days (mating period). Then they were transferred to separate aquaria and observed for about 20 days (postmating period). The snails

deposited their egg-masses on the underside of floating Styrofoam tablets (sometimes on the aquarium wall) 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 about 25°C. As albinism is recessive, the occurrence of fertilization of the albino's eggs by sperm of its melanic mate was ascertained by inspection of its F_1 offspring (presence of eye-spots in cross-bred embryos, absence in selfed ones). 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 egg-masses laid by the melanic mate after separation of the couple were kept in small petri dishes, and the first 50 hatched F_1s (or less, in the case of less fertile Ps) were reared separately in small aquaria up to egg-laying. A series of egg-masses consecutively laid by each of these F_1s was examined for eye-spot recognition in at least 50 embryos. As these isolated 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 parental melanic mate by sperm of the parental albino), and that only melanic F_2s were produced if there had been no cross-fertilization of the F_1 eggs.

Once a crossing experiment was over, each parental albino (which in the present investigation remained not cross-fertilized) was mated with a homozygous melanic *B. straminea* from its own population to control its capacity to be cross-fertilized. As to the melanic parental specimen 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.

As shown by Paraense (1955) in *Biomphalaria glabrata,* a snail inseminated by a conspecific mate of its own or of a near population stores allosperm enough for fertilizing its eggs during a long period (up to two months or even more). Earlier exhaustion of allosperm store is observed in crosses between conspecific individuals of far separated localities, e. g. 1,500 km (Paraense, 1956: 405-406). As the source of albino *B. straminea* used in the crossing experiments (Tangará) is about 1,600 km east by south from Tucuruí, control experiments were carried out by mating 20 pigmented *B. straminea* from Belém (1°27'S, 48°29'W), state of Pará, with 20 albinos from Tangará, which is about 1,600 km east-southeast from Belém.

Voucher specimens have been deposited in the Malacological Collection, Instituto Oswaldo Cruz: *Biomphalaria kuhniana* (Paramaribo, No. 462; Damboentong, No. 971; Tucuruí, Nos. 2913, 2918); *B. straminea* (Tangará, No. 3699; Belém, No. 2653).

RESULTS

The snail from Paramaribo – The shell (Fig. 1) is small, 6.3 mm in diameter, 2.3 mm in width at the aperture (2.1 at the beginning of the outer whorl) and with 4 whorls in the largest specimen, thin, moderately lustrous and translucent, horn-colored. The whorls, loosely coiled, increase rather rapidly in diameter, are rounded on the periphery and on both sides, slightly subangular on the left. Surface with fine growth lines and very faint spiral lines. Suture deeply indented on the left, less so on the right. Right side funnel-shaped, with the first whorl deeply situated and partly hidden by the following whorls. Left side concavity wider and shallower than the right one, and with the first whorl plainly visible. Aperture roundly heart-shaped, usually in the same plane as the body whorl but somewhat deflected to the left in some specimens; peristome sharp; a distinct callus on the parietal wall.

As usual with *Biomphalaria* species, the exposed soft parts are diffusely pigmented with more or less intense gray. The melanin pigment of the pulmonary wall is arranged in irregularly distributed blackish spots, darker, larger and more crowded on the right half, which is more exposed to light. The cephalopodal mass and the pallial organs have no features of special interest. The ventral membrane of the renal tube is even and smooth, showing no renal ridge.

The genital system is shown in Fig. 2. The following description is based on 10 dissected specimens.

The ovotestis diverticula are nearly always simple, rarely bifurcate; 59 simple and 2 bifur-

cate diverticula were found in a minutely dissected ovotestis. The seminal vesicle is relatively well-developed, forming a cluster of predominantly finger-like parietal diverticula preceded and followed by smaller knoblike ones.

The oviduct, the pouch of the oviduct and the nidamental gland have no special characteristics. The vaginal wall is marked with transverse wrinkles to the left of the spermathecal duct, occupying about half the circumference of the vagina. The wrinkles are clearly seen after removal of the fibrous pigmented membrane that clothes the vagina and contiguous organs, preferably using a camel's-hair brush. The wrinkled area is about as long as the spermathecal duct, but sometimes it may reach a higher level, up to about the length of the spermathecal body. The vaginal wall looks like that of *B. straminea*, as described by Paraense & Deslandes (1955) under the name of *Australorbis centimetralis*, but the wrinkles are less developed (compare Figs. 2 and 7). The spermathecal body is usually from obovate to spatulate in outline; its shape, however, may vary more widely according to its state of repletion. The spermiduct, longer than the oviduct to which it is adhered, follows a flexuous course. Soon after emerging from the furrow of the pouch of the oviduct, the spermiduct gives off a series of 4 to 7 prostatic diverticula (mean 5.8 ± 1.03), in most instances divided into 2 to 4 branches which may show knoblike terminal subdivisions. Single diverticula, less frequent, are usually long, slender and the hindmost ones. The foremost prostatic diverticulum is inserted between the body of the spermatheca and the uterine wall. The widest portion of the vas deferens is about half as wide as the penial sheath. The latter, uniformly cylindric in shape, is slender and usually somewhat longer than the prepuce, the ratio between the lengths of the two organs (penial sheath/prepuce) varying from 1.00 to 1.26 (mean 1.15 ± 0.07) in the examined sample. The penis, about as long as the penial sheath, tapers to a point where is the outlet of the penial canal. Histologically it shows a well developed erectile tissue surrounding the penial canal and enveloped by a double muscular coat, of which the inner layer is longitudinal and the outer one circular, the whole invested with the outer epithelium (Fig. 16). As usual with *Biomphalaria*, there are two main extrinsic muscles inserted into the cephalic end of the penial sheath, at its junction with the prepuce: a retractor arising from the columellar muscle and a protractor connected to the head wall. A variable number of smaller retractor and protractor muscles are attached to the preputial wall.

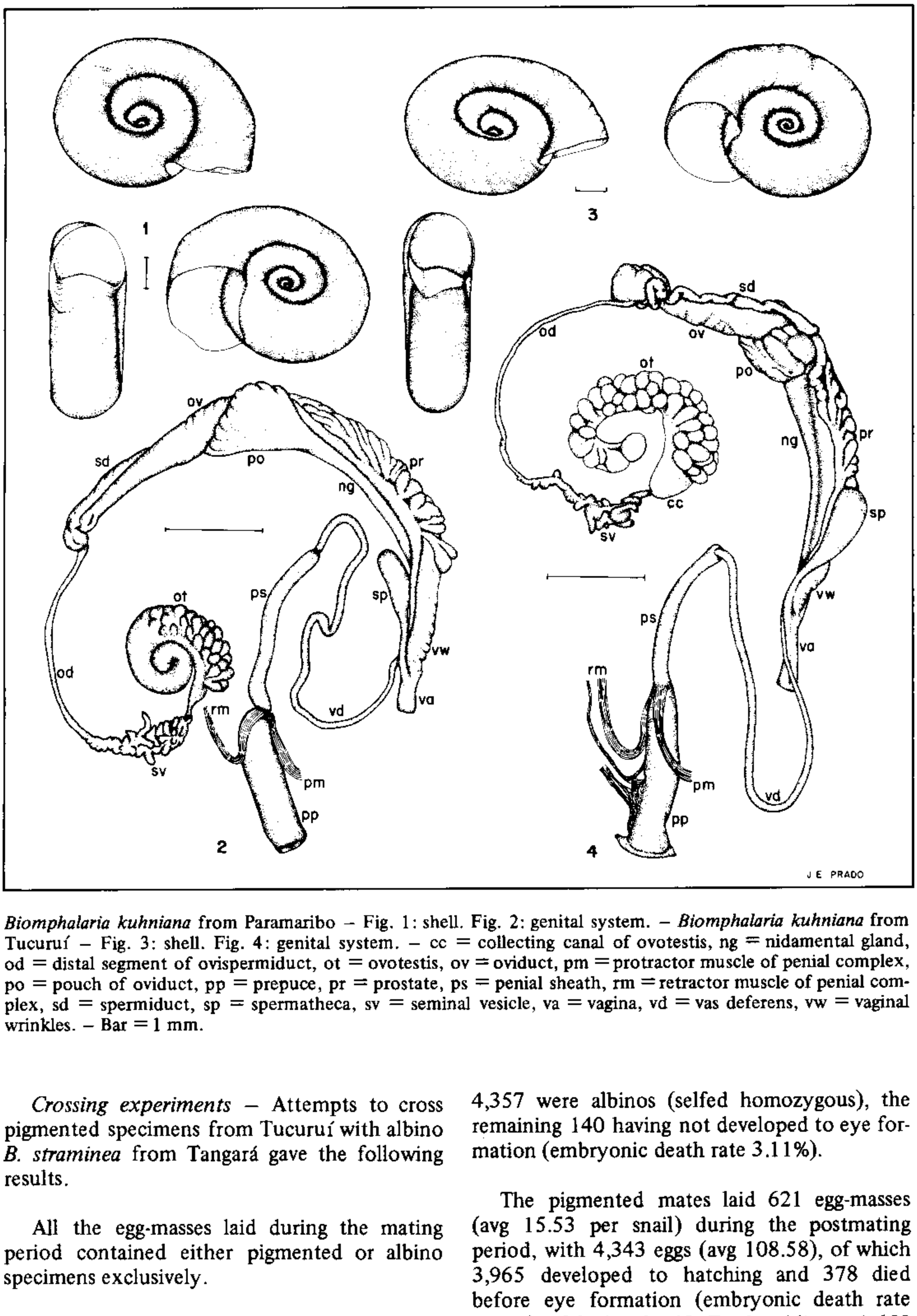
The jaw and the radula do not differ from those of other biomphalarias. The radula of the largest specimen, 6.3 mm in shell diameter, had the formula 18-1-18 and 108 horizontal rows of teeth (Fig. 11).

The specimens from Saramacca – As mentioned above, the specimens from this sample were indistinguishable from those from Paramaribo. Most shells were encrusted to a variable extent with a black coat of ferric oxide (positive Prussian blue reaction). The largest specimen measured 6.3 mm in shell diameter. In five dissected specimens the number of prostatic diverticula varied from 5 to 7, and the penial sheath/prepuce ratio from 1.10 to 1.24.

The snail from Tucuruí – The shell (Fig. 3) is similar to that of the Surinam specimens. The largest one, among about 250 observed, is 7.4 mm in diameter, 2.8 mm in width at the aperture (2.1 mm at the beginning of the outer whorl) and has 4.5 whorls. Anatomically (Fig. 4) they agreed in every respect with those from Surinam. Two minutely dissected ovotestes had 64 and 60 diverticula, with 3 and 4 bifurcate units, respectively. The prostatic diverticula (Fig. 8) varied from 4 to 9 (mean 6.23 ± 1.27), and the penial sheath/prepuce ratio from 0.76 to 1.30 (mean 1.08 ± 0.10); these ranges completely cover the distributions of the respective characters in the Surinam specimens. The penis had the same histological structure as in the Paramaribo snails, and the radula showed no significant differences (Fig. 10).

The following are some data on egg production by Tucuruí snails, based on 500 specimens reared in isolation:

- shell diameter at the onset of egg-laying: 4 mm in 54 specimens, 4.5 mm in 131, 5 mm in 250, 5.5 mm in 39, 6 mm in 26;
- number of eggs in the first-laid egg-mass: 1 (34 specimens) to 15 (1 specimen); mode and median 4 (106 specimens);
- maximum number of eggs per egg-mass during the first month of egg production: 4 (1 specimen) to 20 (1 specimen); mode and median 11 (81 specimens).



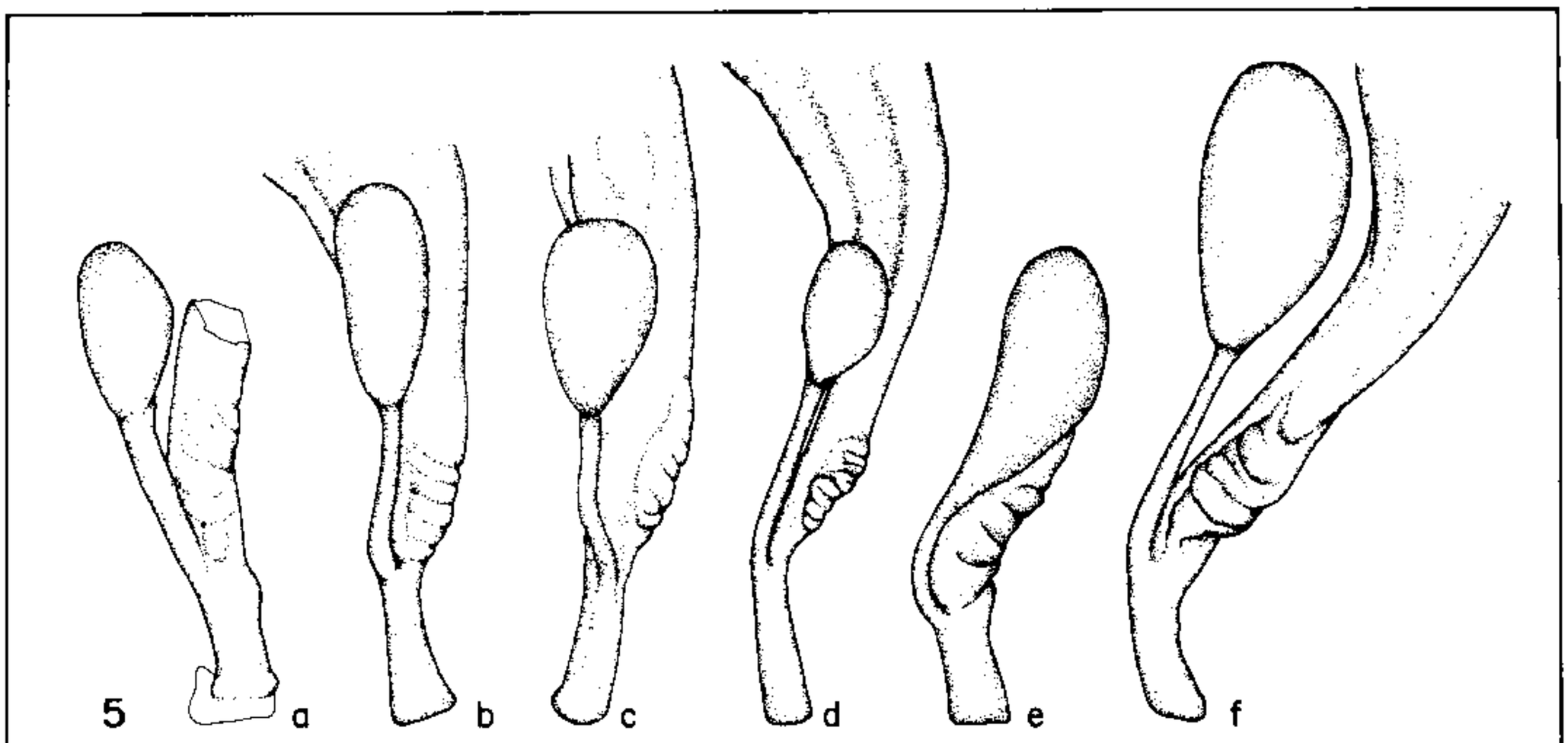
Biomphalaria kuhniana from Paramaribo – Fig. 1: shell. Fig. 2: genital system. – *Biomphalaria kuhniana* from Tucuruí – Fig. 3: shell. Fig. 4: genital system. – cc = collecting canal of ovotestis, ng = nidamental gland, od = distal segment of ovispermiduct, ot = ovotestis, ov = oviduct, pm = protractor 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, vw = vaginal wrinkles. – Bar = 1 mm.

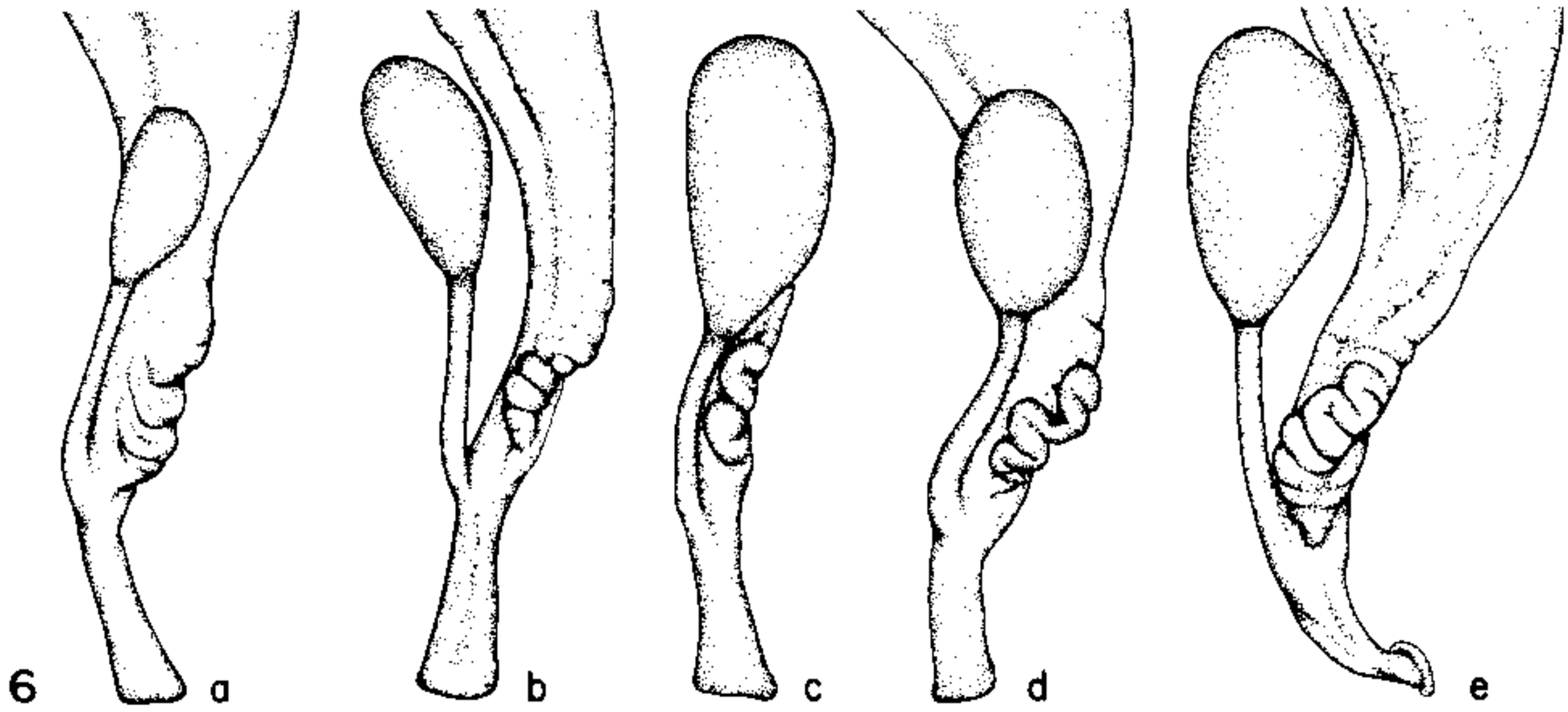
Crossing experiments – Attempts to cross pigmented specimens from Tucuruí with albino *B. straminea* from Tangará gave the following results.

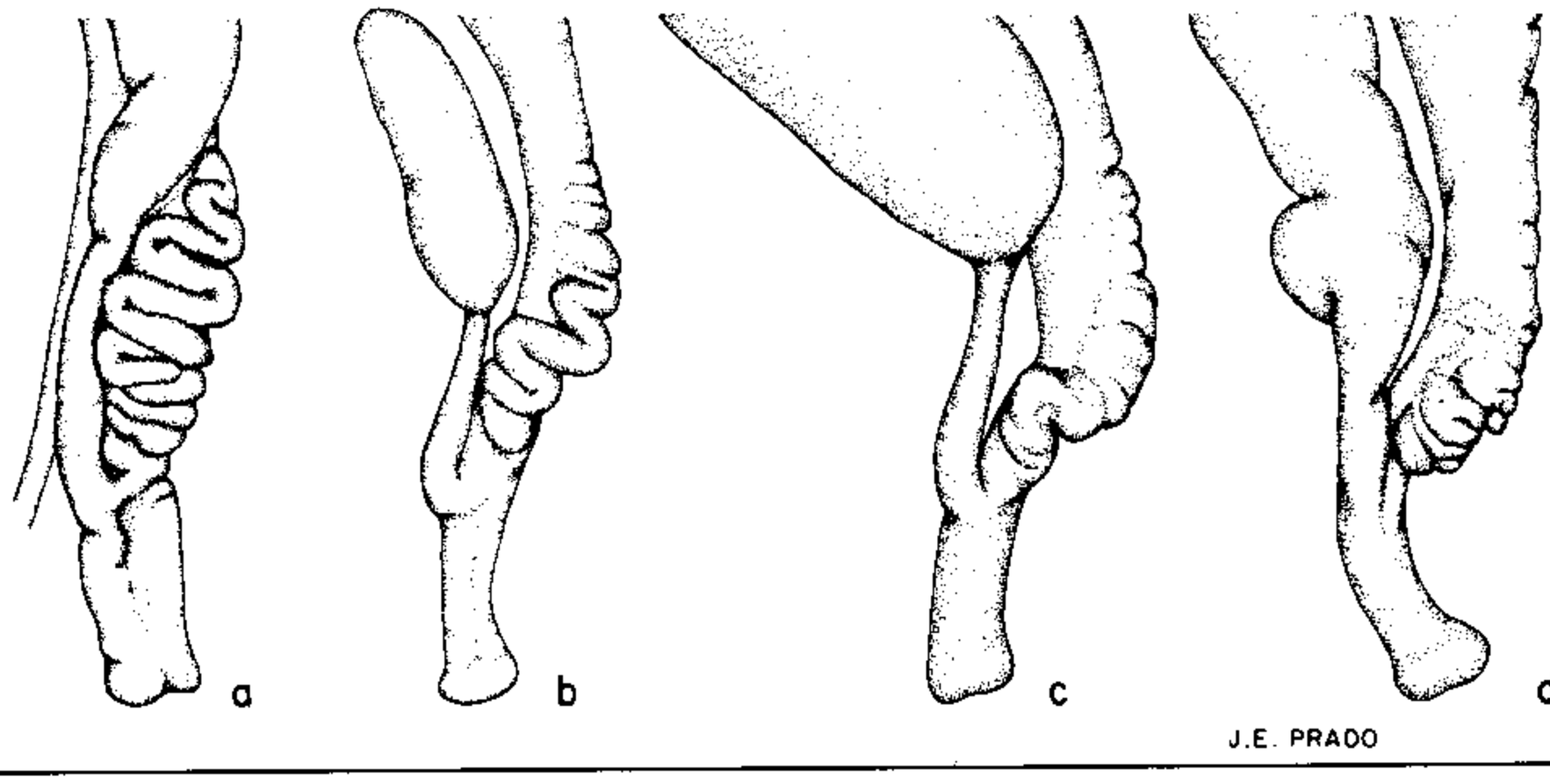
All the egg-masses laid during the mating period contained either pigmented or albino specimens exclusively.

After separation of the couples the 40 albinos laid 548 egg-masses (average 13.70 per snail) with 4,507 eggs (avg 112.68), of which 4,357 were albinos (selfed homozygous), the remaining 140 having not developed to eye formation (embryonic death rate 3.11%).

The pigmented mates laid 621 egg-masses (avg 15.53 per snail) during the postmating period, with 4,343 eggs (avg 108.58), of which 3,965 developed to hatching and 378 died before eye formation (embryonic death rate 8.70%). Of the 3,965 F_1 hatchlings, 1,658 (representing the offspring of 30 pigmented mates which contributed 50 F_1s each, and 10







Biomphalaria kuhniana from Tucuruí – Figs. 5a-f, 6a-e: diversity of vaginal corrugation. – *Biomphalaria straminea* from Tangará – Figs. 7a-d: diversity of vaginal corrugation. – Bar = 1 mm.

which produced 4 to 39 F_1s) were followed to the F_2 generation. All of them proved homozygous, since they produced exclusively black-eyed offspring.

Each of the parental albinos (not fertilized by the pigmented mates from Tucuruí) was readily cross-fertilized when paired with a pigmented *B. straminea* from its own population.

Three specimens from Tucuruí used in the crossing experiments showed anomalies in the male system. One was aphallic, the descending loop of its vas deferens ending blindly beside the vagina; and two were biphallic, the two prepuces fusing distally. All the other specimens looked normal.

The control experiments with pigmented *B. straminea* from Belém and albino ones from Tangará resulted in reciprocal crosses between the 20 couples, according to the previously described pattern (Paraense, 1956: 405-406).

Biomphalaria straminea *from Tangará* – The population from Tangará shows the characteristics described for *B. straminea* (as *Australorbis centimetralis*, a junior synonym) by Paraense & Deslandes (1955). Owing to its great resemblance to the Surinam and Tucuruí snails, the following account will mainly refer to those features that point to some degree of unlikeness.

The shell of the largest dissected snail was 8.5 mm in diameter by 3.1 mm in width at the aperture (2.3 mm at the beginning of the outer whorl), and had 4.75 whorls, but specimens up to about 11 mm in diameter have been found in the population.

The minutely dissected ovotestes of three specimens had 53, 57 and 71 diverticula, with 4, 2 and 3 bifurcate units, respectively. The vaginal wall is markedly corrugated in the great majority of specimens (Figs. 7a, b), there being a few ones in which the corrugation is more or less attenuated (Fig. 7c). In one specimen (Fig. 7d) the vaginal wall had an abnormal appearance. The prostatic diverticula varied from 9 to 18 (mean 13.78 ± 2.09); they are comparatively longer and more branched than in the Surinam and Tucuruí specimens (Fig. 9). The ratio penial sheath/prepuce varied from 0.92 to 1.93 (mean 1.43 ± 0.23). Cross-sections from the middle portion of the penis show that the muscular coat of its wall consists of three layers, whose fibers are disposed longitudinally in the inner one, circularly in the middle one, and obliquely in the outer (Fig. 17).

The following data on egg production are based on 250 specimens reared in isolation:

- shell diameter at the onset of egg-laying: 4.5 mm in 7 specimens, 5 mm in 36, 5.5 mm in 65, 6 mm in 100, 6.5 mm in 29, 7 mm in 13;
- number of eggs in the first-laid egg-mass: 1 (in 74 specimens) to 18 (in 3); mode 1 (74 specimens), median 3 (30 specimens);
- maximum number of eggs per egg-mass during the first month of egg production: 1 (2 specimens) to 30 (1 specimen); mode 22 (18 specimens), median 15 (20 specimens).

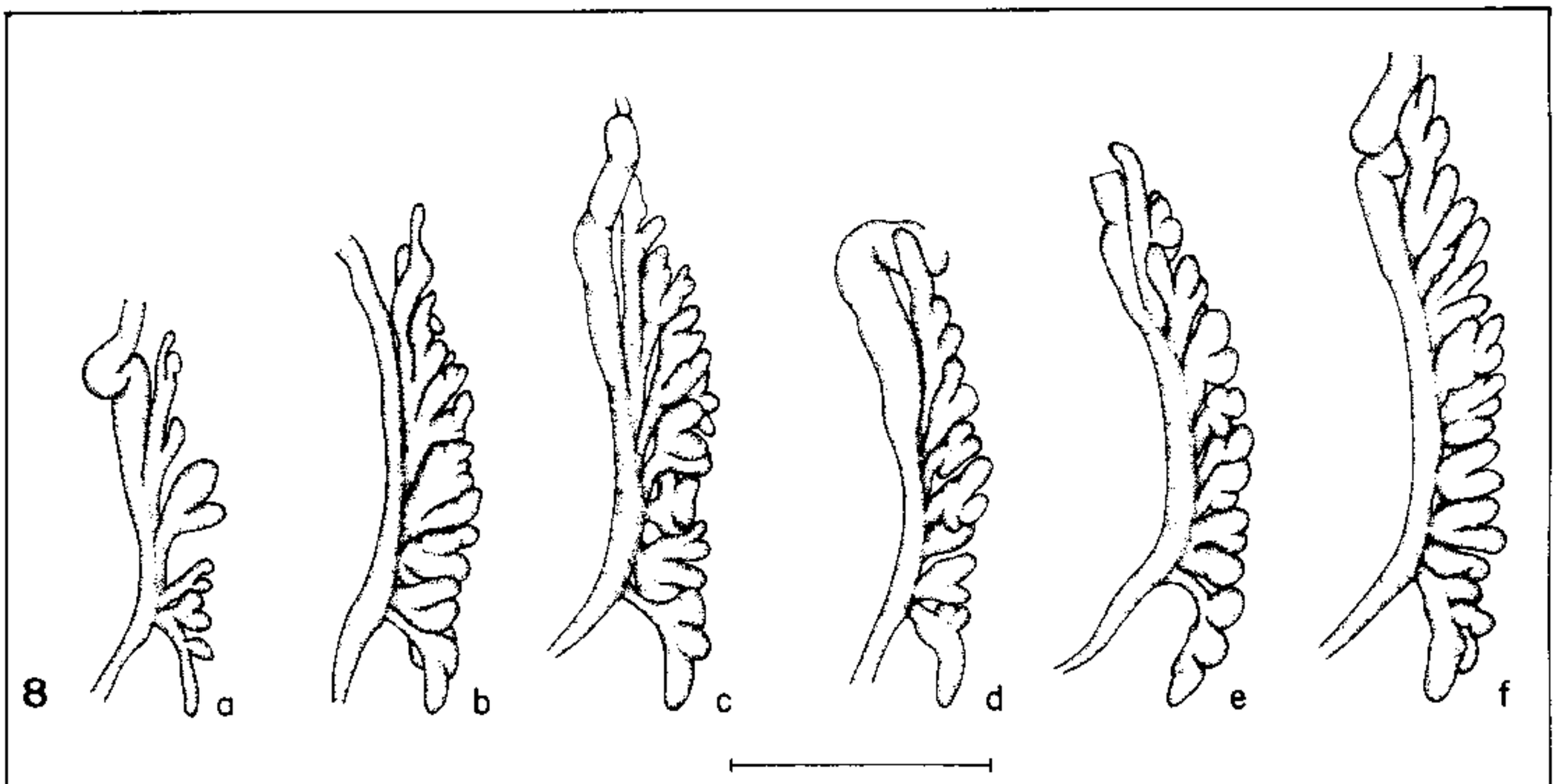
DISCUSSION

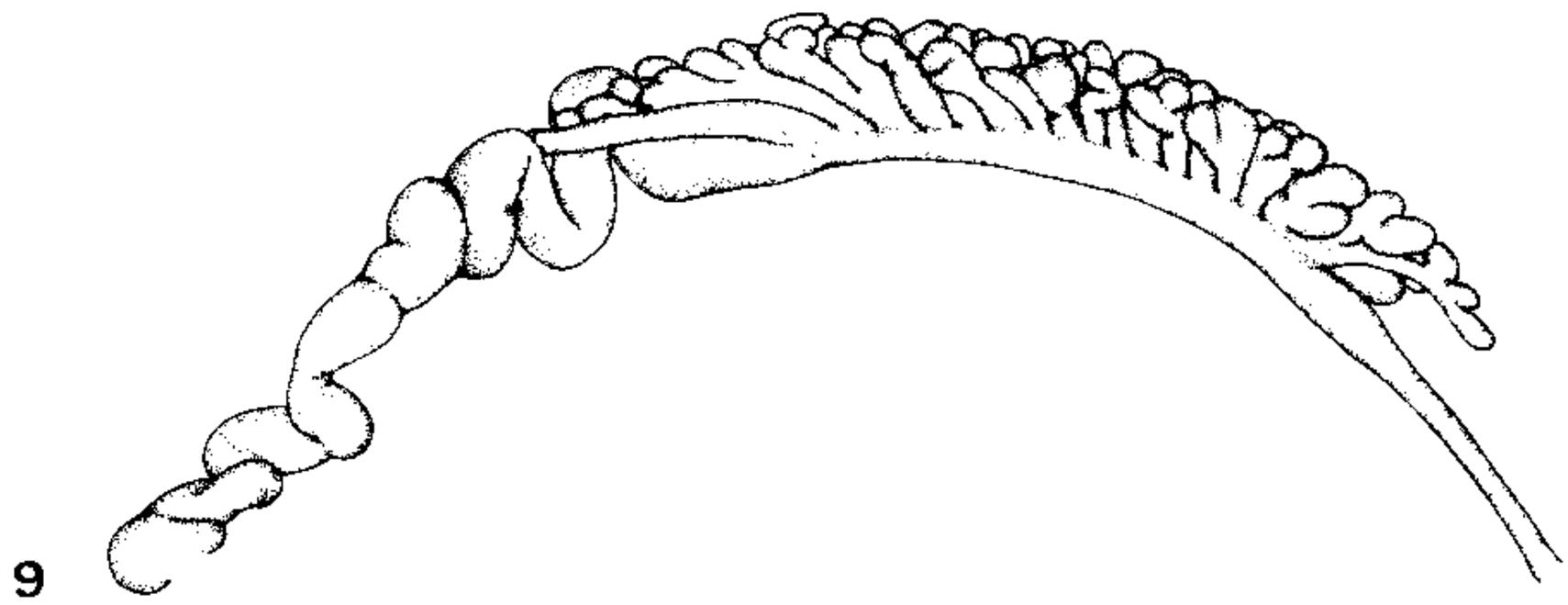
Complete reproductive isolation between the snails from Tucuruí and *B. straminea* from Tangará justifies considering the former a distinct species. Its morphological likeness to the specimens from Surinam, the type locality of *B. kuhniana*, points to its identity with the latter.

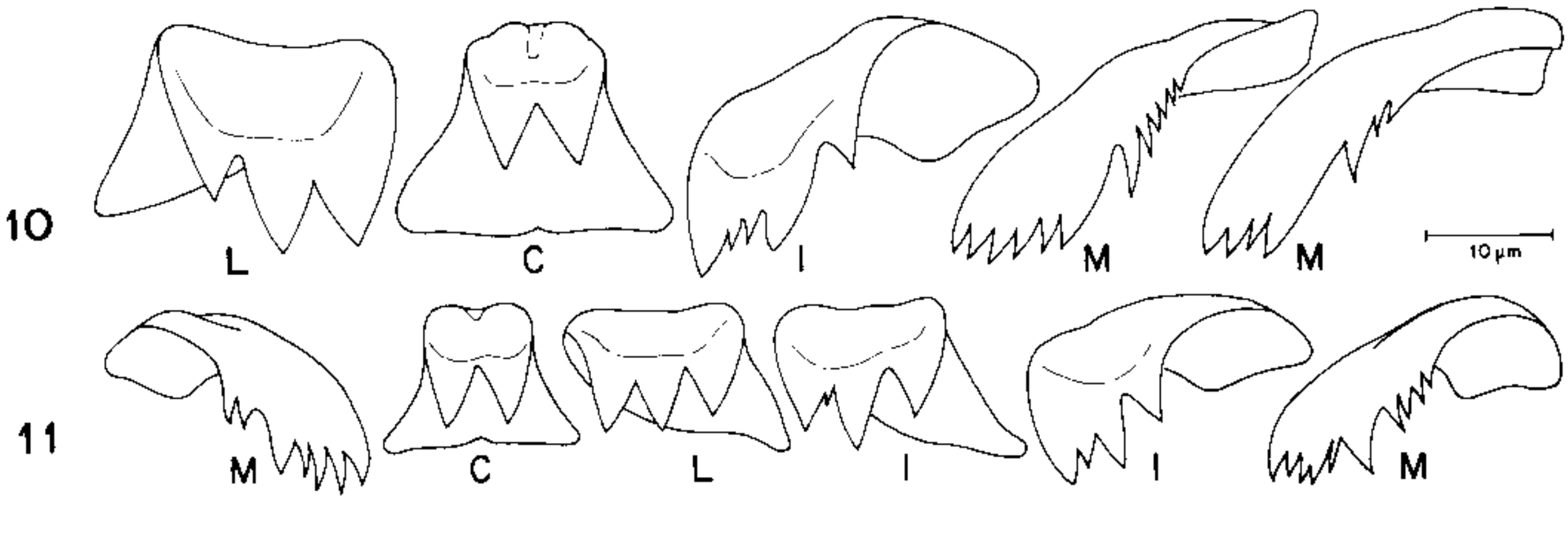
Discrimination between *B. kuhniana* and *B. straminea* on morphological grounds is not always an easy undertaking, chiefly for one who has not had the opportunity of examining a great number of individuals of both species.

The adult shell is larger in *B. straminea*, reaching about 10 mm in diameter in most populations, although specimens up to 16.5 mm are found in our collection. In *B. kuhniana* it scarcely exceeds 7 mm. This character, however, has a limited value owing to the difficulty in recognizing full-grown shells in planorbids, chiefly in small-sized species.

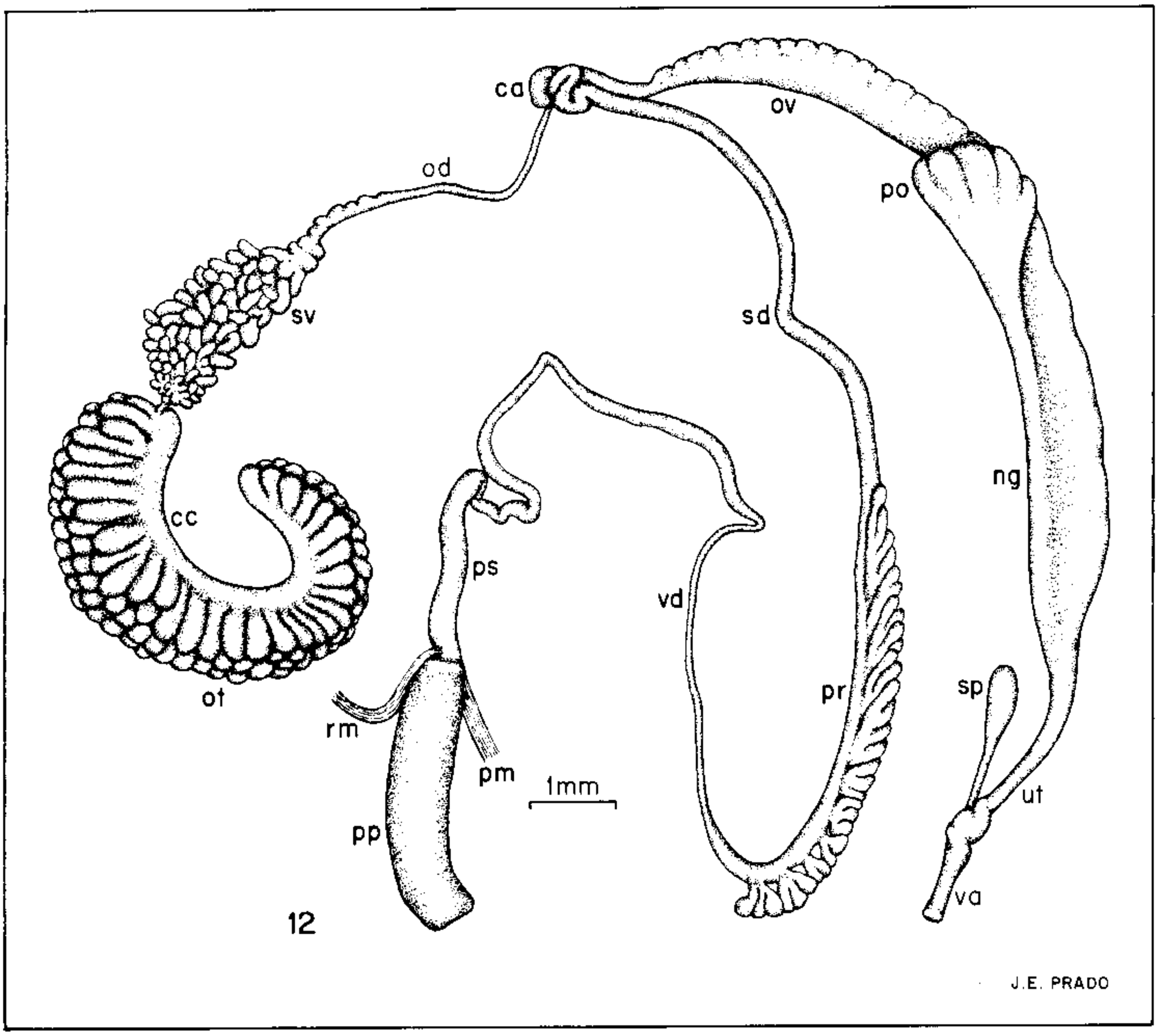
Of the anatomic characters, the vaginal corrugation is less developed in *kuhniana*, consisting of lower, narrower ridges, looking sometimes almost effaced (Figs. 5a, b), but it may show, in some specimens, a gradation of increasing wrinkles, as shown in Fig. 5c-f, 6a-e. On the other hand, the well-developed corrugation of *straminea* (Fig. 7a, b) may look less conspicuous in some specimens, especially frail-bodied ones (Fig. 7c). Although little







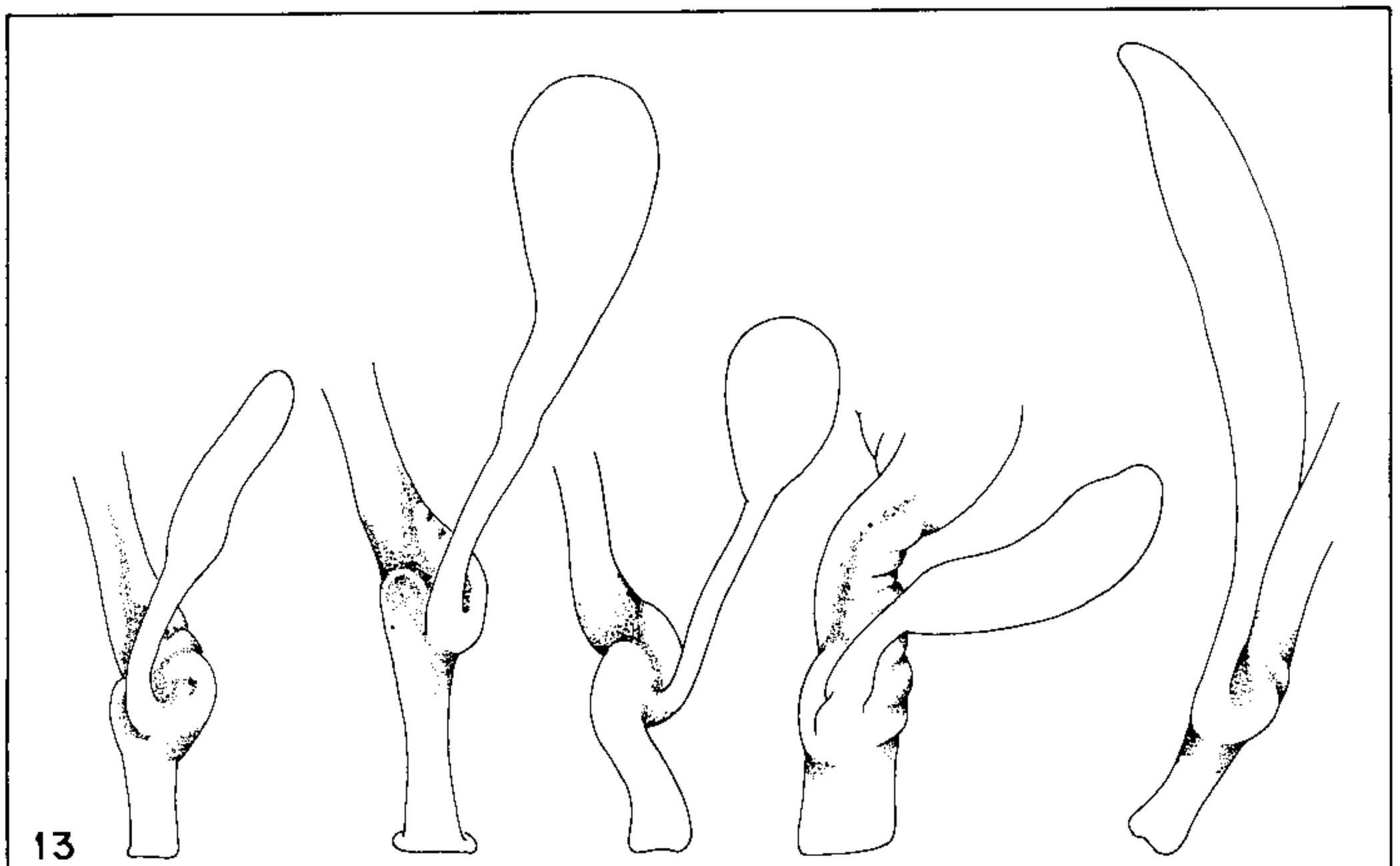
Figs. 8a-f: prostate of *Biomphalaria kuhniana* from Tucuruí. Fig. 9: prostate of *Biomphalaria straminea* from Tangará. – Bar = 1 mm. – Fig. 10: radular teeth of *Biomphalaria kuhniana* from Tucuruí. Fig. 11: radular teeth of *B. kuhniana* from Paramaribo. C = central, I = intermediate, L = lateral, M = marginal.



Biomphalaria intermedia – Fig. 12: genital system. – ca = carrefour; other abbreviations as in Fig. 4.

frequent, those instances of extreme variation (stronger wrinkles in *kuhniana,* slighter ones in *straminea*) may confuse the observer, who ought to resort to other characters in the same specimen or to additional specimens in the sample. Other characters are the number and disposition of the prostatic diverticula and the number of muscle layers in the penis. The prostatic diverticula varied from 4 to 9 (mean 6.23 ± 1.27) in *kuhniana,* and from 9 to 18 (mean 13.78 ± 2.09) in *straminea.* Nine diverticula were found in only two specimens of *kuhniana* and one of *straminea.* The difference between the means is highly significant ($p < 0.001$, t test). The prostatic diverticula are somewhat longer and more branched in *straminea.* The penis, cross-sectioned at its middle, shows two muscle layers in *kuhniana* (inner longitudinal, outer circular), and three in *straminea* (inner longitudinal, middle circular, outer oblique).

Another feature that may prove useful as an auxiliary characteristic is the course followed by the distal segment of the spermiduct (between the pouch of the oviduct and the hindmost prostatic diverticulum): straight or slightly wavy in *kuhniana,* more or less curly in *straminea* (compare Figs. 8 and 9). The ratio penial sheath/prepuce has no diagnostic value, since its distribution in both species widely overlaps. In the present samples this ratio was 0.76 to 1.30 for *kuhniana* and 0.92 to 1.93 for *straminea.* Except for two *kuhniana* specimens with 0.76 and 0.88, all the others overlapped with *straminea;* of the latter, 26 exceeded 1.30, which suggests that a ratio above 1.40 may exclude *kuhniana.*



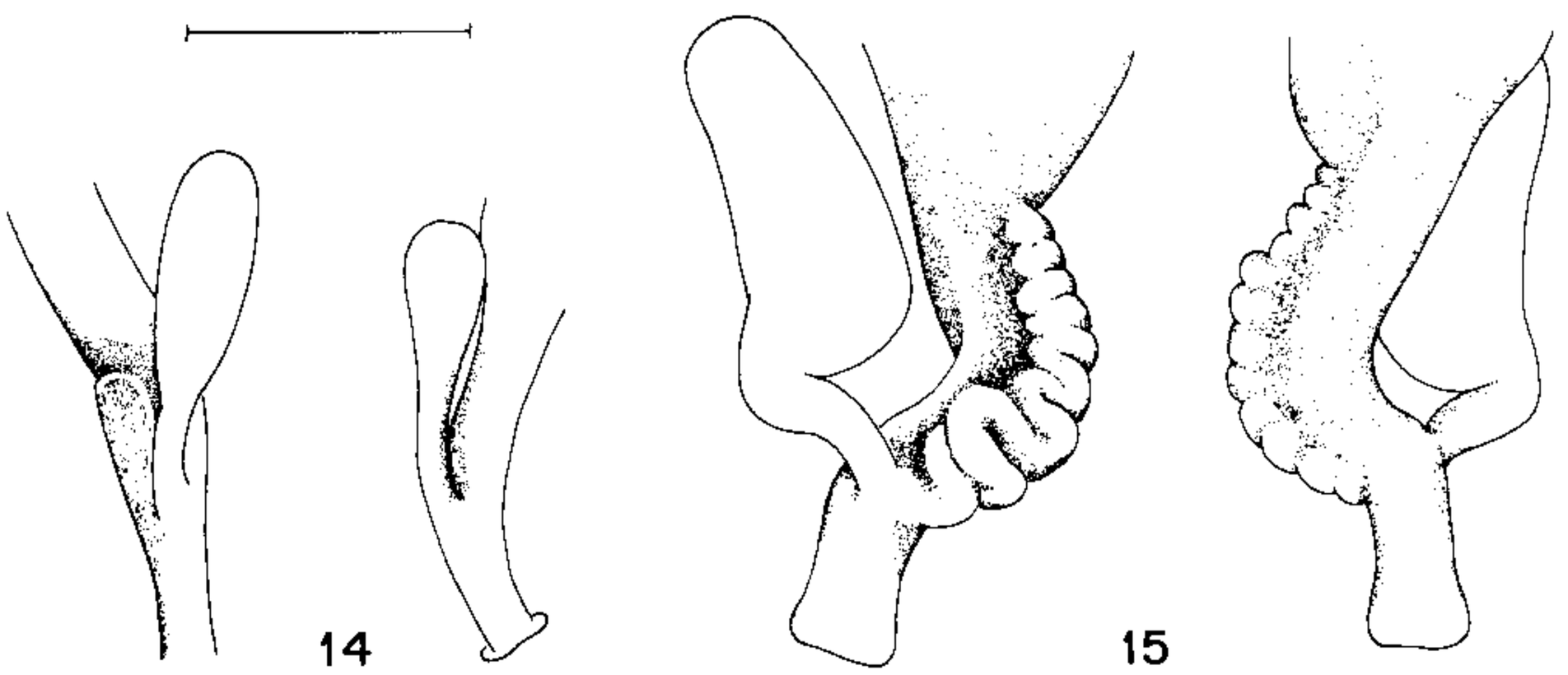
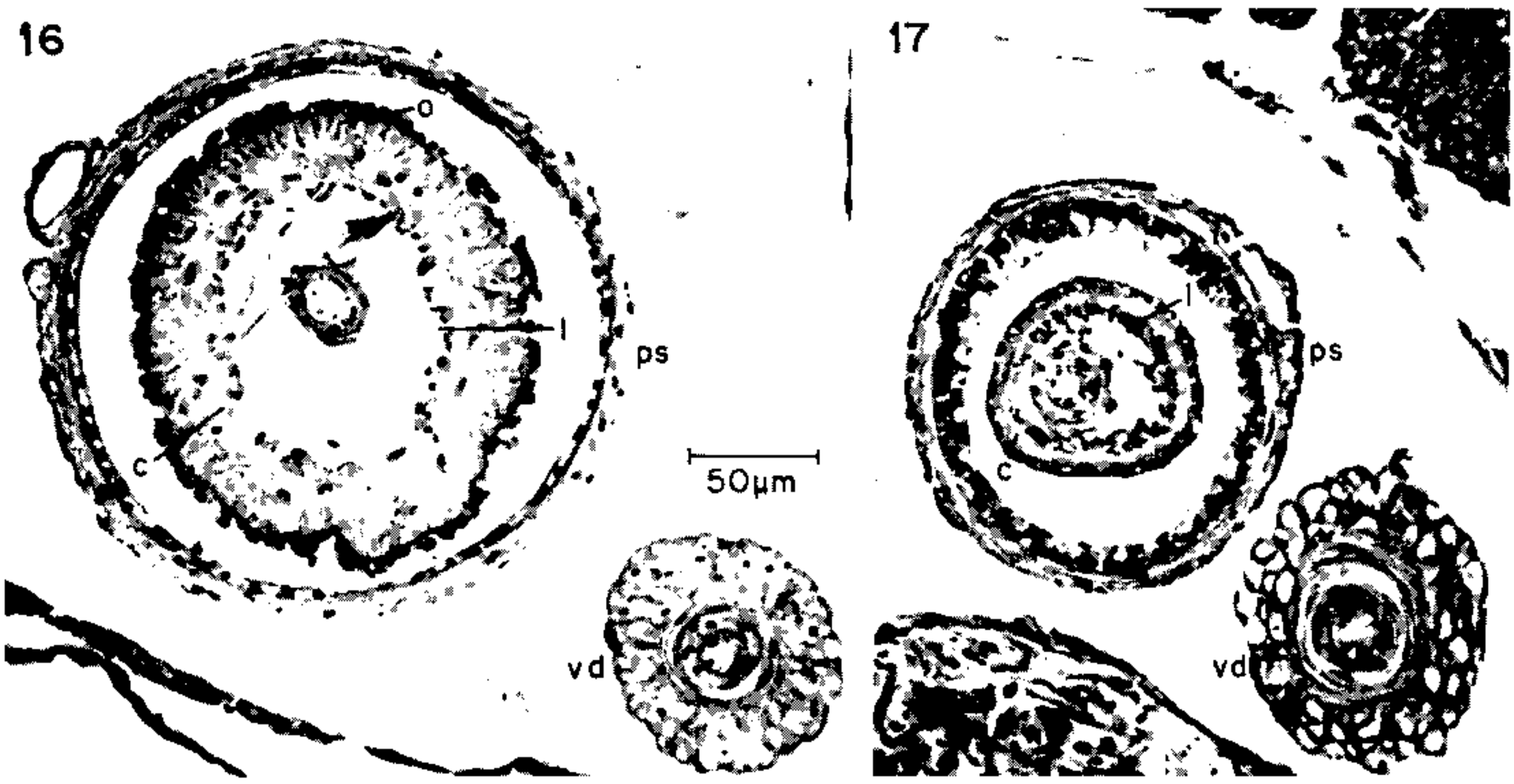


Fig. 13: diversity of vaginal surface in *Biomphalaria intermedia*. Fig. 14: vaginal pouch in *Biomphalaria peregrina*, for comparison with Fig. 13. Fig. 15: vaginal corrugation in *Biomphalaria straminea*, for comparison with Fig. 13. – Bar = 1 mm.

Another species that may be confused with *B. kuhniana* is *B. intermedia* (Paraense & Deslandes, 1962). According to present knowledge, their ranges are separated by a huge gap between parallels 5°S and 20°S, corresponding to about 1,700 km. The shell is very alike in both species, and the difference between them is to be sought in the aspect of the vagina. Whereas in *kuhniana* the vaginal wall shows the usually weak corrugation described above, and the rest of its surface is smooth, in *intermedia* there is usually a combination of a more or less developed corrugation (or sometimes a mere swelling) on the left of the spermathecal duct (to the right of the observer) and a rudimentary pouch on the right of the duct (more properly,



Cross-sections through middle portion of penis in *Biomphalaria kuhniana* (Fig. 16) and *B. straminea* (Fig. 17). – c = circular muscle layer, l = longitudinal muscle layer, o = oblique muscle layer, ps = penial sheath, vd = distal (ascending) segment of vas deferens.

on the columellar side of the vagina). Both corrugation and pouch, however, never reach the degree of development observed in *B. straminea* and *B. peregrina*, respectively (compare Figs. 12, 13, 14, 15). The number of prostatic diverticula varies from 6 to 20 in *intermedia* (8 to 11 in about 75% of the specimens), and the ratio penial sheath/prepuce from 0.5 to 1.7, thus having no diagnostic value. The penis has two layers of muscle fibers arranged as in *kuhniana*. Summing up, discrimination of *kuhniana* and *intermedia* has to be based on careful examination of the vaginal region, assisted in lucky situations by a number of prostatic diverticula above 9.

Since its description by Clessin (1883), *B. kuhniana* has been identified exclusively by shell characters (Vernhout, 1914; F. C. Baker, 1945; Floch & Lajudie, 1945; Floch & Fauran, 1954a, b). H. B. Baker (1930) described the jaw and the radula of a specimen from Bejuma (Venezuela), whose characteristics are the same as in the other biomphalarias. Strangely enough, he placed *kuhniana* under *Planorbula*, a genus restricted to North America, whose chief characteristics are a set of apertural lamellae within the shell aperture and a preputial gland which are absent in this species; he distinguished *kuhniana* from *straminea* by a single shell character, the smaller size of the former.

As our knowledge of Neotropical biomphalarias increases, we are led to the recognition of sibling species that can be grouped into species complexes. It is evident from the present study that a *Biomphalaria straminea* complex may be recognized, including *straminea*, *kuhniana* and *intermedia*. Biochemical studies would perhaps confirm this statement.

RESUMO

Biomphalaria kuhniana **(Clessin, 1883), molusco planorbídeo da América do Sul** – A validade de *Biomphalaria kuhniana* (Clessin, 1883) é confirmada pelo estudo morfológico de espécimes do Suriname (localidade tipo) e da área de Tucuruí (rio Tocantins, Estado do Pará, Brasil), em comparação com *B. straminea* (Dunker, 1848), e por experiências de cruzamento que revelaram completo isolamento reprodutivo entre as duas espécies. A concha adulta de *kuhniana* é menor (cerca de 7.5 mm) que a de *straminea* (11 mm a 16.5 mm). As duas espécies distinguem-se anatomicamente pelo grau de enrugamento da parede vaginal (pouco desenvolvido em *kuhniana*, conspícuo em *straminea*), pelo número e aspecto dos divertículos prostáticos (em *kuhniana* 4 a 9, mais curtos e menos ramificados; em *straminea* 9 a 18, mais longos e mais ramificados), pelo

número de camadas musculares na parte média do pênis (duas em *kuhniana*, três em *straminea*), e pelo percurso do segmento distal do espermiduto, geralmente direto ou ligeiramente ondulado em *kuhniana*, mais ou menos enroscado em *straminea*.

As diferenças entre *B. kuhniana* e *B. intermedia* (Paraense & Deslandes, 1962) são menos acentuadas. Na *B. intermedia* a concha atinge cerca de 12 mm de diâmetro, a próstata tem 7 a 15 divertículos, há duas camadas musculares na parte média do pênis e a parede vaginal apresenta um enrugamento mais ou menos desenvolvido (ou às vezes uma simples dilatação) à esquerda do canal da espermateca e uma bolsa rudimentar à direita do canal.

Em vista da grande semelhança morfológica entre *B. straminea*, *B. kuhniana* e *B. intermedia*, é proposto o agrupamento das três espécies no complexo *Biomphalaria straminea*.

Palavras-chave: Mollusca – Planorbidae – *Biomphalaria kuhniana* – *Biomphalaria straminea* – *Biomphalaria intermedia* – espécies crípticas – taxionomia

ACKNOWLEDGEMENTS

To Dr. D. C. Geijskes, Director (1959) of the Surinaams Museum, for working facilities, and to Piet Bolwerk, of the Museum staff, for assistance in collecting in Paramaribo; to ELETRONORTE, for supporting the field work in the area of Tucuruí.

REFERENCES

BAKER, F. C., 1945. *The molluscan family Planorbidae*. xxxvi + 530 p. Univ. Illinois Press, Urbana.

BAKER, H. B., 1930. The mollusca collected by the University of Michigan-Williamson expedition in Venezuela. *Occ. Papers Museum Zool. Univ. Michigan*, No. 210: 1-95.

CLESSIN, S., 1883-1886. Die Familie der Limnaeiden enthaltend die Genera *Planorbis, Limnaeus, Physa* und *Amphipeplea*, p. 29-430. *In* MARTINI & CHEMNITZ, *Systematisches Conchylien-Cabinet*, Bauer & Raspe, Nürnberg.

FLOCH, H. & FAURAN, P., 1954a. Bilharziose intestinale et *Tropicorbis kühnianus* (Clessin) en Guyane Française. *Arch. Inst. Pasteur Guyane Française*, Publ. No. 325: 1-7.

FLOCH, H. & FAURAN, P., 1954b. Essais infructueux d'infection expérimentale de *Tropicorbis kühnianus* (Clessin) par *Schistosoma mansoni*. *Bull. Soc. Pathol. Exot., 47* (3): 452-459.

FLOCH, H. & LAJUDIE, P., 1945. Sur les bilharzioses en Guyane Française. *Inst. Pasteur Guyane Française et Inini*, Publ. No. 119: 1-5.

HARRY, H. W., 1962. A critical catalogue of the nominal genera and species of Neotropical Planorbidae. *Malacologia, 1* (1): 33-53.

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., 1963. The nomenclature of Brazilian planorbids. III. *Australorbis stramineus* (Dunker, 1848). *Rev. Brasil. Biol., 23* (1): 1-7.

PARAENSE, W. L. & DESLANDES, N., 1955. Studies on *Australorbis centimetralis*. I. Morphology, in comparison with *A. glabratus*. *Rev. Brasil. Biol., 15* (3): 293-307.

PARAENSE, W. L. & DESLANDES, N., 1962. *Australorbis intermedius* sp. n. from Brazil (Pulmonata, Planorbidae). *Rev. Brasil. Biol., 12* (4): 343-350.

RANSON, G., 1955. Observations préliminaires sur quelques Planorbes américains. *Bull. Muséum Hist. Nat.*, Sér. 2e, *27* (3): 220-225.

VERNHOUT, J. H., 1914. The non-marine molluscs of Surinam. *Notes Leyden Museum, 36:* 1-46.