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The influence of varied relaxing and fixing conditions on anatomical characters in a Planorbis species

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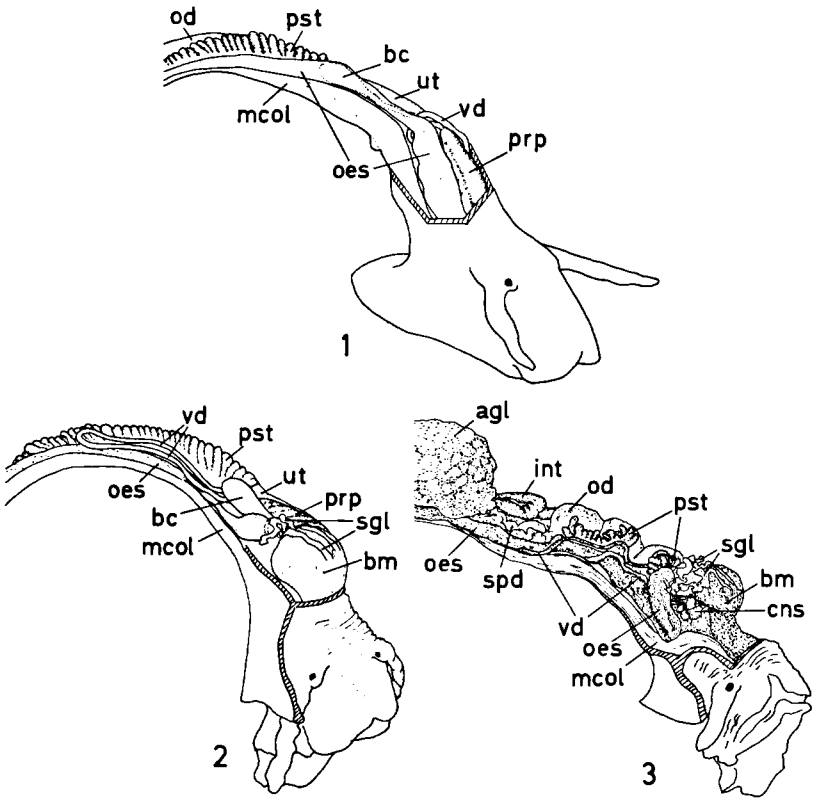
It has become a common practice to include quantifiable data in taxonomical studies, e.g., ratios of dimensions. The effect of different contractility of molluscan soft parts, however, has usually not been examined. In order to fill this gap for one group, the genus *Planorbis*, snails fixed without relaxation were compared with those in two different stages of relaxation.

MATERIAL AND METHODS

A stock of *Planorbis intermixtus* Mousson, 1874, was kindly provided by Dr. J. Massoud, Teheran, who collected the snails in the Caspian Sea area of North Iran. The stock has been maintained in the laboratory in Tübingen. The three fixing states were obtained as follows.

1. Fully extended snails.

Animals were relaxed with a mixture of at least 5 mg pentobarbital in 10 ml of water or a 0.05 and 0.1% solution of nembutal for 30 hours and fixed in a 4% formaldehyde solution (cf. Meier-Brook, 1976)



Figs. 1-3. Position and condition of reproductive organs in a fully extended (1), a half retracted (2), and a fully contracted snail (3). Key to lettering: agl – albumen gland, bc – bursa copulatrix, bm – buccal mass, cns – central nervous system, int – intestine, mcol – columellar muscle, od – oviduct, oes – oesophagus, prp – preputium, pst – prostate gland, sgl – salivary glands, spd – sperm duct, ut – uterus, vd – vas deferens.

before preservation in 70% alcohol. The snail's cephalopedal mass is extended as if the animal were moving (fig. 1).

2. Incompletely extended or half-retracted snails.

Animals were insufficiently relaxed before fixation, either by shortening the relaxing time of by inadequate concentrations of the relaxing agent (Meier-Brook, 1976, table 2). The cephalopedal mass is retracted

to immediately behind or inside the aperture, the sole of the foot being bent and folded (fig. 2).

3. Fully retracted and shrunken snails.

Animals were transferred from the aquaria into 70% alcohol immediately. The cephalopedal mass is retracted into the shell by about 1/3 to 1/2 of a whorl (fig. 3). Organs are contracted and jammed (e.g., prostate gland, = pst in fig. 3) as far backwards as the columellar muscle reaches, viz., to the region of the albumen gland and the stomach. All parts behind remain unaffected by the method of fixation. Form and dimensions of the kidney are altered. Its length is usually shortened by approximately one half in animals killed in alcohol without relaxation as compared with fully relaxed animals. The hind part of the kidney (the "saccular portion" in Baker, 1945) is swollen.

RESULTS

Concerning the characters of the reproductive system usually employed in taxonomy the results are shown in table 1. The only organ changing dimensions appeared to be the preputium which was contracted in length – and consequently enlarged in width – by 36% on an average in fully contracted snails when compared with that in fully extended individuals. Even in incompletely relaxed animals ("half retracted") the preputium is significantly shortened. The penis sheath on the other hand does not show significant alterations in length though it can be shown to be contractile too in living snails. Likewise the lengths of the vas deferens (v.d.) and the free sperm duct (free spd) do not vary significantly from relaxed to unrelaxed individuals.

Calculating ratios of dimensions meets the argument that absolute measurements vary with growth and that the material studied is usually not homogeneous with respect to size. A ratio widely used in anatomical studies is that of penis sheath/preputium. Table 1 shows that these figures are significantly increased in fully contracted, but even in half retracted, individuals due to shortening of preputia, whereas differences in the ratios v.d./p.sh. are not significant.

The length of the spermoviduct does not differ depending on the relaxation and fixation, but the length of the seminal vesicula is an unreliable parameter in any case and thus not suitable for comparison, because under tension applied during dissection it can reach more than twice the length of the organ in resting stage.

state of fixation	length of preputium		t-test	length of penis sheath		t-test
	n*	$\bar{x}$ ± s.d.	p	n*	$\bar{x}$ ± s.d.	p
a) fully extended	19	2.28 ± 0.86	<0.01 in a/b	16	1.55 ± 0.17	>0.05 in a/b
b) half retracted	13	1.57 ± 0.29	>0.05 in b/c	13	1.42 ± 0.37	>0.05 in b/c
c) fully contracted	20	1.45 ± 0.33	<0.001 in a/c	21	1.49 ± 0.22	>0.05 in a/c

state of fixation	ratio $\frac{\text{penis sheath}}{\text{preputium}}$			t-test	ratio $\frac{\text{vas deferens}}{\text{penis sheath}}$			t-test
	n*	range	$\bar{x}$ ± s.d.	p	n*	range	$\bar{x}$ ± s.d.	p
a) fully extended	16	0.28–0.90	0.75 ± 0.18	<0.05 in a/b	14	1.31–4.31	3.27 ± 0.73	>0.05 in a/b
b) half retracted	12	0.64–1.27	0.93 ± 0.22	<0.05 in b/c	10	1.73–5.64	3.62 ± 1.02	>0.05 in b/c
c) fully contracted	20	0.57–1.46	1.07 ± 0.22	<0.001 in a/c	21	2.22–5.33	3.39 ± 0.77	>0.05 in a/c

Table 1. Lengths (in mm) and length ratios of reproductive organs of *Planorbis intermixtus* in various states of fixation.

* Differences in numbers of individuals were caused by the fact that sometimes organs broke during dissection or could not be measured due to inappropriate fixation.

CONCLUSIONS

In *Planorbis intermixtus* the preputium is the only part of the genital system which changes its dimensions from fully extended to half or fully retracted individuals so considerably that absolute dimensions or ratios calculated from them should be compared only if the state of contraction is the same. The penis sheath, vas deferens, and free sperm duct as well as the number of prostate diverticula of course are not subject to variation caused by different relaxing or fixing conditions.

Since in the genus *Planorbis* s.s. interspecific variation in anatomical characters is low, this should also apply to other species, as *Planorbis planorbis* (L.) and *P. carinatus* O.F. Müller. In most other planorbid genera conditions might be different and should be examined prior to anatomical studies. Thus in many groups the muscular layer of the penis sheath is much stronger than in *Planorbis* and must be expected to contract after the stimulus of fixation without relaxation.

SUMMARY

Dimensions and length ratios of reproductive organs in *Planorbis intermixtus* Mousson were compared in snails fixed after complete and incomplete relaxation with pentobarbital or nembutal and in snails killed in alcohol without relaxation. The only organ significantly changing its length is the preputium. Its dimensions should be used in taxonomy only when the fixing conditions are the same. Any other parts of the reproductive system including the penis sheath in this group do not differ significantly between animals killed with or without relaxation.

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