COMPARATIVE RADULAR MORPHOLOGY OF *LYMNAEA (BULLASTRA) CUMINGIANA* (PULMONATA: LYMNAEIDAE) AND RELATED TAXA IN THE INDO-PACIFIC REGION

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Abstract. The radular morphology of *Lymnaea (Bullastra) cumingiana* was compared to that of five other Indo-Pacific lymnaeid "species", namely: *L. (Radix) quadrasi* (Philippines), *L. (R.) rubiginosa* (Indonesia and Thailand) and *L. (R.) viridix*(Guam and Hong Kong) in order to investigate the taxonomic relationship among the six species. Although all six species uniformly exhibited a unicuspid, slightly asymmetrical central (rachidian) tooth and tricuspid laterals, interesting differences were noted among the outer marginals. These were observed to be uniquely bicuspid in *L. cumingiana,* predominantly tricuspid in *L. quadrasi,* tetracuspid in *L. rubiginosa* (Indonesia and Thailand) and multicuspid in *L. viridis* (Guam and Hong Kong). Thus, the results support the hypotheses that *L. cumingiana* is a unique species compared to the rest, that *L. quadrasi* is closely related to *L. rubiginosa* (Indonesia and Thailand) and that the two geographical isolates of *L. viridis* have not diverged. Radular morphology was therefore found to have a limited significance in elucidating the taxonomic relationship between the six groups of lymnaeids studied.

INTRODUCTION

Traditional molluscan taxonomy has relied mainly on comparative morphology. This has particularly been the case for the family Lymnaeidae whose classification has presented some of the most confusing problems (Wright, 1971). The problem arises due to the extremely great morphological range of variation within the species and the great morphological uniformity as well, within the genus *Lymnaea* in its entirety (Hubendick, 1951).

The typical lymnaeid shell is dextral and ovately long and may be turreted to broad-oval. The spire is more or less attenuated and varies considerably in height. The columellar axis is typically gyrate (spiraled) or twisted. The shell varies in thickness and in size of body whorl (which is generally wide). The umbilicus may be open or closed and sutures are distinct. The aperture is wide and has a sharp fragile peristome. Most species have thin yellowish brown or yellowish green shells with rounded whorls.

Internally, the organs are similarly dextrally coiled. The mantle edge is plain and smooth. Tentacles are flattened and triangular rather than filiform. Gills and pseudobranches are absent. The kidney is large and pear-shaped while the ureter proceeds directly forward without flexure (Benthem Jutting, 1956; Malek, 1962; Malek and Cheng, 1974).

The radula is considered an organ of taxonomic significance. The number, shape, size and position of the cusps on the central, lateral and marginal teeth are important taxonomic characteristics (Malek and Cheng, 1974). It was therefore the objective of this paper to investigate if comparative radular morphology can be used to determine the taxonomic relationship between the endemic Philippine species, *Lymnaea (Bullastra) cumingiana,* and five other lymnaeid "species" in the Indo-Pacific region, namely: *L. (Radix) quadrasi* (Philippines), *L. (R.) rubiginosa* (Indonesia and Thailand) and *L. (R.) viridis* (Guam and Hong Kong).

MATERIALS AND METHODS

All six lymnaeid "species" in this study were raised in the snail room of the Applied Malaco-

Table 1

Characteristics of the radula of the six lymnaeid species under study.

Radular character examined	Snail species					
	L. cumingiana	*L. quadrasi*	*L. rubiginosa* (I)	*L. rubiginosa* (T)	*L. viridis* (G)	*L. viridis* (HK)
1. central or rachidian tooth	unicuspid, slightly asymmetrical	unicuspid, slightly asymmetrical	unicuspid, slightly asymmetrical	unicuspid, slightly asymetrical	unicuspid, slightly asymetrical	unicuspid, slightly asymetrical
2. lateral teeth	irregularly tricuspid	irregularly tricuspid	irregularly tricuspid	irregularly tricuspid	irregularly tricuspid	irregularly tricuspid
3. marginal teeth:						
a) inner	tricuspid	tricuspid	tricuspid	tricuspid	tricuspid	tricuspid
b) outer	bicuspid*	tricuspid* and tetracuspid	tetracuspid* and multicuspid	tetracuspid* and multicuspid	tetracuspid and multicuspid*	tetracuspid and multicuspid*
4. dental formula	20 : 13 : 1 : 13 : 20	25 : 11 : 1 : 11 : 25	14 : 8 : 1 : 8 : 14	18 : 8 : 1 : 8 : 18	13 : 9 : 1 : 9 : 13	21 : 9 : 1 : 9 : 21
5. cusp formula	2 : 3 : 1 : 3 : 2	3 : 3 : 1 : 3 : 3	4 : 3 : 1 : 3 : 4	4 : 3 : 1 : 3 : 4	4 : 3 : 1 : 3 : 4	4 : 3 : 1 : 3 : 4

* = dominant type
multicuspid = with 5 or more cusps

logy Center, Department of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok. Radular mounts were prepared for study and comparison according to modified methods described by Bowell (1915), Verdcourt (1948) and Meeuse (1950).

The buccal mass of the snail was removed by dissection then boiled in 2.5% NaOH for 1-2 minutes in a small test tube. The contents of the tube were emptied into a watch glass and the radular ribbon removed under the dissecting microscope. If brittle, the radula was soaked in a drop of 0.1% glacial acetic acid to restore pliability. The radula was then washed in 3 changes of distilled water, 3 minutes per rinse. This was followed by staining in a depression slide with a 4% solution of eosin Y in 95% ethanol, controlled under the microscope. Excess stain was rinsed off rapidly.

While moist, the radula was flattened on a slide with needles so that the cusps faced upwards. It was covered with a coverslip, small weights (20-30 g) were added and allowed to dry. When dry, the slide was tapped so that the coverslip fell off leaving the radula attached to the slide. It was finally mounted in Permount.

RESULTS

Radulae of the six species under study were studied under both low (LPO) and high power (HPO) objectives of the microscope. Due to their minute size, the oil immersion (OI) objective was necessary for studying radulae obtained from *Lymnaea viridis* (Guam and Hong Kong). Important radular characteristics are summarized in Table 1 while camera lucida drawings of representative radular teeth are presented in Fig 1.

All six species had a small unicuspid, slightly asymmetrical central (rachidian) tooth which was slightly askew to the right. Similarly, the laterals were all irregularly tricuspid and consisted of a small short internal cusp situated close and adjacent to a much larger and longer medial cusp, both of which seemed fused opposite on overhanging outer cusp. The number of laterals on each side of the central tooth seemed quite consistent for each species: 13 for *Lymnaea cumingiana,* 11 for *L. quadrasi,* 8 for *L. rubiginosa* (Indonesia and Thailand) and 9 for *L. viridis* (Guam and Hong Kong).

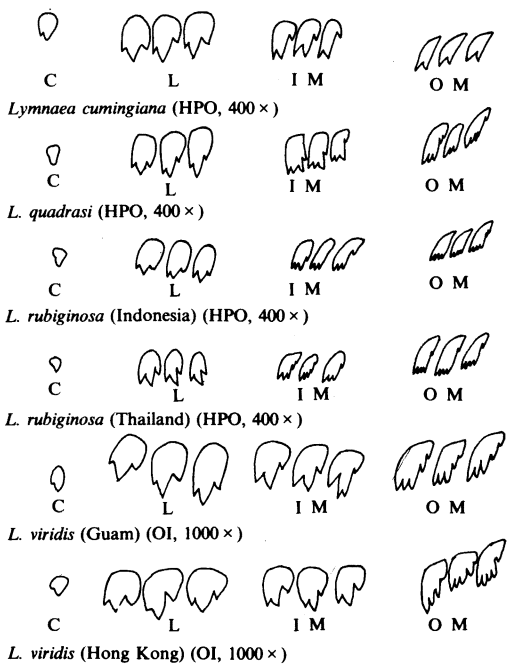

Fig 1—Camera lucida drawings of radular teeth for each of the six lymnaeid species under study. (C = central, L = lateral, IM = inner marginal, OM = outer marginal)

In contrast, the number of marginals did not seem to be a consistent characteristic. The number recorded was found to vary according to the level of the radular ribbon at which the counting was done and the size of the specimen studied. It was observed that the number of marginal teeth decreased towards the anterior extremity of the ribbon and likewise among the smaller specimens. Thus, measurements were obtained as much as possible from the central region of the radula in average-sized specimens.

The marginal teeth could be classified into two unclearly delineated types, the inner and outer marginals. The inner marginals, situated immediately after the laterals, were all tricuspid but in various types of "transitional" forms, *ie* they began to metamorphose from the lateral into the constant outer marginal pattern. It was therefore often difficult to determine precisely where the laterals ended and the marginals began.

Nevertheless, these outer marginals turned out to have more diagnostic value in differentiating

the six species. *Lymnaea cumingiana* had inner marginals whose outer cusp slowly diminished in size giving way to the dominant bicuspid condition among the outer marginals. These bicuspid marginals were unique for this species and set it apart from the rest. *L. quadrasi* on the other hand had both tri- and tetracuspid marginals although the tricuspid form was more dominant.

L. rubiginosa (Indonesia) and *L. rubiginosa* (Thailand) were predictably similar in having predominantly tetracuspid and occasionally multicuspid (with > 5 cusps) marginals. Lastly, *L. viridis* (Guam) and *L. viridis* (Hong Kong) had marginals similar to those of *L. rubiginosa* but differed however in being smaller and predominantly multicuspid.

It was found that the processed radulae were better kept as semi-permanent mounts using absolute alcohol as the medium. Permount was found to obscure the teeth of some specimens, making them opaque and indistinguishable after a few days.

DISCUSSION

Hubendick (1951) stated that, other than the radula, "the remaining parts of the alimentary system, buccal bulb, oesophagus, gizzard, the intestine and its gland, have, in my experience, no variations of comparative morphological value". Thus, the radula is generally regarded as the most important taxonomical feature of the alimentary system. However, on account of the small size of the radula and of the extension of its parts through different optical levels during microscopic examination, it is very difficult to avoid mistakes of observation, as confirmed by the fact that several authors have overlooked the asymmetrical structure of the centrals in the lymnaeid radula.

In this study, radular morphology was found to have a limited significance in differentiation of the six species from each other. The pulmonate radula is generally of a primitive type with numerous small and comparatively simple teeth of fairly similar shape in each cross row (Hubendick, 1978).

The unicuspid central and tricuspid lateral teeth of all species studied were morphologically similar, differing only in their smaller size in the case of *Lymnaea viridis* (Guam and Hong Kong). However, the typically bicuspid marginals clearly separated *L. cumingiana* from the rest of the group; throughout the Lymnaeidae, this feature has only been observed in *L. cumingiana* and *L. buruana* (Hubendick, 1951). *L. quadrasi* could also be distinguished from *L. rubiginosa* (Indonesia and Thailand) by the predominance of tricuspid outer marginals in the former compared to tetracuspids in the latter. In contrast, the smaller species, *L. viridis* (Guam and Hong Kong) tended to have marginals which were predominantly multicuspid (wish 5 or more cusps).

The number of lateral teeth in a given row seemed to be a relatively consistent and specific character although this should be verified in the future by studying a larger sample size for each species. There were wider variations in the number of marginals per row depending on the region of the radula where the teeth were counted and the age and size of the specimen. However, Hubendick (1945) proved that the radula grows not only by increasing the number of transversal and longitudinal rows but also by increasing size of the teeth. Furthermore, teeth belonging to a certain longitudinal row gradually pass over from the marginal into the lateral type concurrently with the issue of new transverse rows.

Thus, Hubendick (1951) believed that it was inappropriate to lay much stress on radular structure with regards to comparative anatomy of lymnaeids. His views agree with those of other workers.

Annandale and Rao (1925) wrote, "The structure of the jaws and radulae is, with a few exceptions, remarkably uniform in the Indian Limnaeidae and in many species individual variability is sufficiently great to mask any specific characters that may occur" while Roszkowski (1929) proposed "As it is known, systematists have placed great hopes in the radula, thinking that the differences in its structure will enable them to distinguish not only families and genera, but even species. Personally I think that the facts have not justified such hopes, and the importance of the radula as a systematics character is greatly exaggerated".

Thus, the results support the hypotheses that *Lymnaea cumingiana* is a unique species compared to the rest, that *L. quadrasi* is closely related to *L. rubiginosa* (Indonesia and Thailand) and that the

two geographical isolates of *L. viridis* have not diverged. Comparative radular morphology was therefore found to have a limited significance in elucidating the taxonomic relationship between the six groups of lymnaeids studied.

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