

Chapter 3

Materials and methods

Mangrove areas in the upper gulf of Thailand (in Trat, Chantaburi, Rayong, Chonburi, Chachoengsao, Samutprakan, Bangkok, Samutsakon, Samutsongkram, and Phetchaburi Provinces) were surveyed. Snails were collected from June 1998 to January 2000 by eyes searching from each type of mangrove trees as well as on mud surfaces. Specimens were collected from different habitats for completely malacofauna species. Collectings have been done in triplicate sets both in wet (May to October) and dry (November to April) season.

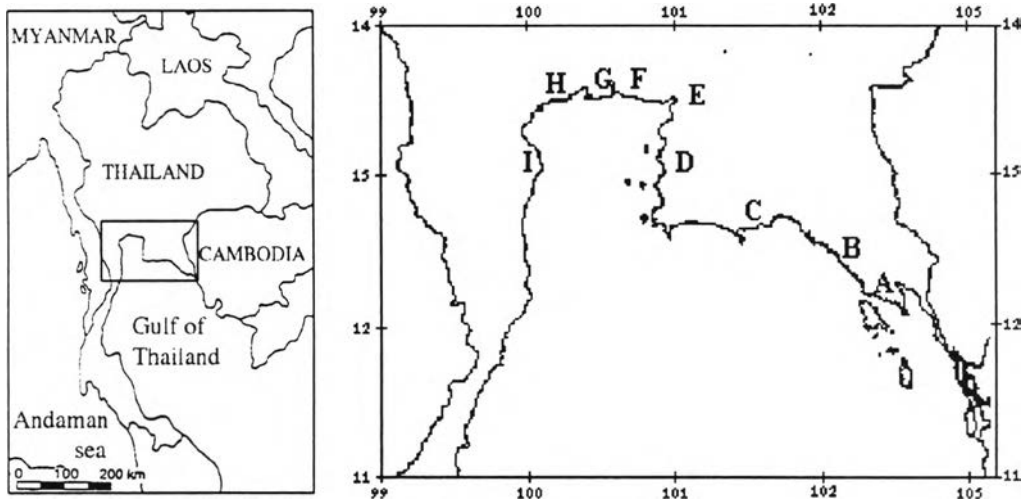


Fig. 3-1 Map of the upper Gulf of Thailand indicating the mangrove areas of study (A, Trat; B, Chantaburi; C, Rayong; D, Chonburi; E, Chachoengsao; F, Samutprakan; G, Bangkok; H, Samutsongkram; I, Phetchaburi).

Specimens were suffocated in bottle for 24 hrs and preserved in 70% ethanol. The large size specimens (shell or body length are more than 6-8 cm) were injected by 95% ethanol to their body cavity. Specimens were labeled for recorded including the collection number, scientific name, locality, collected date, collector, number of specimens and habitats.

Specimens were identified by comparison with Brandt's collection at Faculty of Tropical Medicine, Mahidol University and Raffle Museum, National University of Singapore and using

the according main literatures, Britton, 1984, Brandt 1974, Habe, 1964, Hubendick, B. 1964, and Vermeulen and Whitten, 1998

Shell morphology of ellobiids and ampibolids including shell length (SL), shell width (SW), body whorl length (BWL), aperture length (AL), aperture width (AW), and spire length (SRL) were measured in millimeters. These parameters are illustrated in figure 3-2.

- shell length is measured from apex to base of shell,
- shell width is measured from widest part of shell,
- body whorl length is measured from last whorl to base of shell,
- aperture length is measured from upper most of aperture to base of aperture,
- aperture width is measured from center of columella to each margin of aperture in vertical plan and
- spire length is calculated from shell length minus body whorl length.

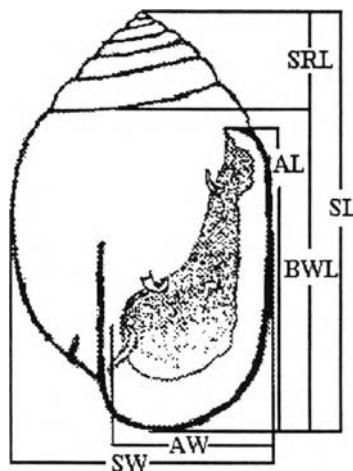


Fig. 3-2 Terminology of shell measurement of Ellobiidae and Amphibolidae using shell of genus *Cassidula* as a representative.

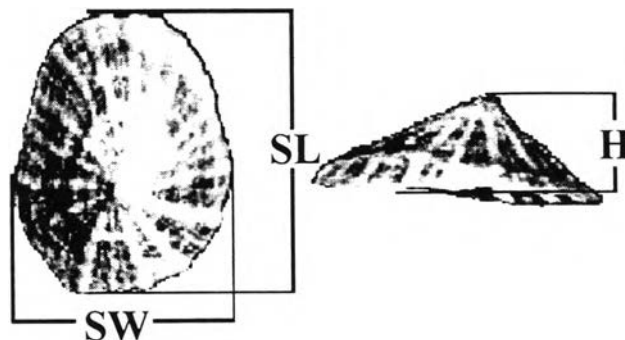


Fig. 3-3 Terminology of shell measurement of Siphonariidae

Shell morphology of siphonariid (shell length; SL, shell width; SW, and shell height; H) were measured. Shell length is a distance from anterior to posterior margin; shell width is a distance from left to right margin and shell height is a distance from apex to the base of shell.

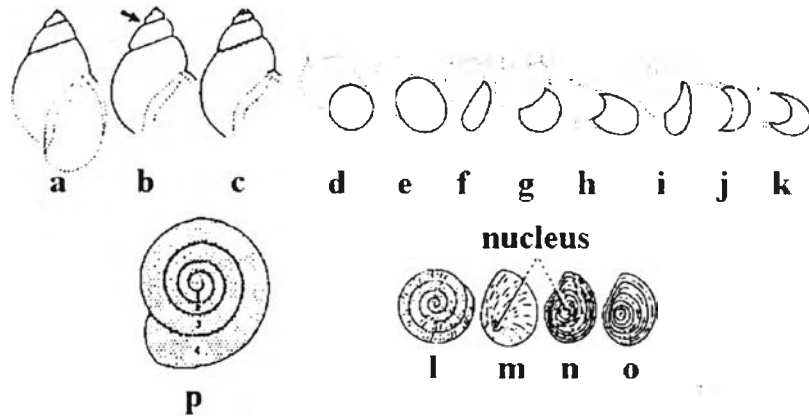


Fig. 3-4 Shell terminology. A, suture slightly indented; b, suture strongly indented; c, crenulate sutures; d, round aperture; e, oval aperture; f, narrowly oval aperture; g, roundly lunate aperture; h, ovate-lunate aperture; i, narrowly ovate-lunate aperture; j, broadly lunate aperture; k, deeply lunate aperture; l, multispiral operculum; m, paucispiral operculum; n, concentric operculum; o, concentric operculum with spiral nucleus; p, method of counting whorls (After Burch, 1962).

External morphology of onchidiid (body length; BL , body width; BW , foot length; FL , and foot width; FW) were measured as follows; body length is measured by anterior to posterior body edge, body width is measured by left to right body edge, foot length is measured by anterior to posterior foot edge and foot width is measured by left to right foot edge.

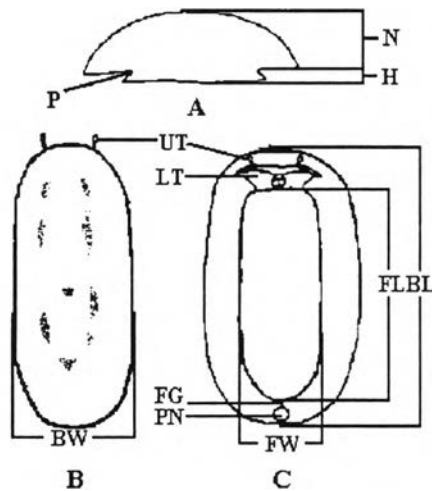


Fig. 3-5 Morphological terminology of measurement of Onchidiidae A) cross section of body B) dorsal and C) ventral view.

Radulae were removed from buccal masses and placed in warm 10% NaOH. They were washed in water and dehydrated in 95% ethanol, stained in lactophenol blue then washed in absolute ethanol before clearing in xylene and mount in Canada Balsum. Radula teeth types were investigated and counted each rows under microscope and formula arrangement were determined.

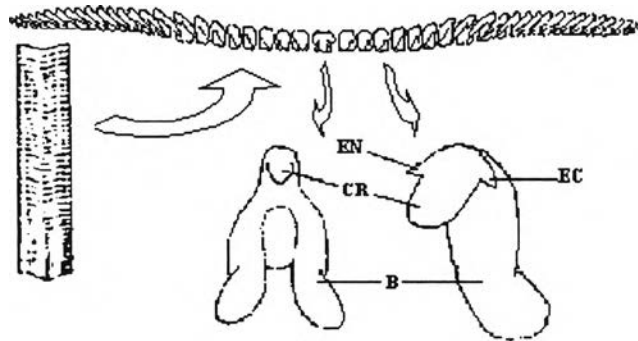


Fig. 3-6 Terminology of radula. Above is a single row of radular teeth, below left is radular sheath, and the two rights are central and lateral teeth (B, base; CR, crown; EN, endocone; EC, ectocone).

The whole animals were dissected under dissecting microscope for separation of reproductive organ, stomach, and nerve ganglia. Each organ was drawn, scaling and then kept in 70% ethanol respectively. Uncleared penis and nerve ganglia samples were stained by methylene blue solution before drawing. Collection number and number of dissected specimens and radulae are shown in appendix III.

Nine characters and character states were selected and based on Martins (1996) and Tillier (1989) for phylogenic reconstruction by heuristic method in PAUP (Swofford, 1991). Outgroup is *Haminoeo symnistra*, opisthobranch in mangrove forest.

Table 3-1 List of characters used to reconstruct the phylogenic relationship. Character 1-6 refer from Martins (1996a) and Character 6-9 refer from Tillier (1989)

NO.	Character	Character states	Type	Weight
1	Auly	0 = monauly 1 = incipient semidiauly 2 = advanced semidiauly 3 = diauly	Ordered	4
2	Glandular cover of pallial gonoducts	0 = entirely covered 1 = partly covered 2 = naked	Ordered	4
3	Position of insertion of bursa duct	0 = near female genital opening 1 = on anterior third of oviduct 2 = on posterior third of oviduct	Ordered	3
4	Status of sperm groove	0 = opened 1 = closed	Ordered	4
5	Parietovisceral connectives	0 = <0.90 1 = 0.90-1.9 2 = 2.0-4.0 3 = >4.0	Unordered	1
6	chiastoneury	0 = present 1 = absent	Ordered	4
7	Length of cerebral commissure (CC)	1 = greater than 1.1 x right cerebral ganglion width 2 = between 1.1-0.9 x right cerebral ganglion width 3 = less than 0.9 x right cerebral ganglion width	Ordered	1
8	Length of the right cerebro-pedal connective (CPD)	1 = longer than twice the width of the right cerebral ganglion 2 = between 1-2 times right cerebral ganglion width 3 = shorter than right cerebral ganglion width	Ordered	1
9	Ratio between the lengths of the cerebro-pedal connectives (Left /right) (CPR)	1 = less than 0.9 2 = from 0.9-1.1 3 = from 1.1-1.5 4 = from 1.5-2.5	Ordered	1