CHAPTER 2
MATERIALS AND METHODS

2.1 Introduction

This chapter provides the research design and methodology necessary to accomplish the objective of the study which is primarily to determine the types and diversity of monogeneans on the freshwater catfish of Thailand. The sites and methods of fish collections, the methods used for the collections and preparation of monogeneans for taxonomic studies as well as the methods used for the morphological analysis of the monogeneans obtained (categorisation of sclerotised parts, characterisations of the monogeneans and the use of cluster analysis to group the different monogeneans in the form of dendrograms of relationships) are given in this Chapter.

2.2 Fish collection

To achieve the objective of this study it was deemed necessary to collect and examine all the 98 catfish species known to occur in Thailand (Vidthayanon, unpublished data) for monogeneans. However, due to time and manpower constraints, and because some fish species are rare and endangered, only 44 fish species could be collected in this study

In most cases natural (feral) populations of catfishes were examined for monogeneans, however, some species of catfish (*Hemibagrus nemurus*, *Pangasianodon gigas* and *P. hypophthalmus*) were obtained from fish farms. Besides the indigenous catfish species, imported catfish such *Clarias gariepinus* cultured in farms were also examined for monogeneans.

2.2.1 Fish collection sites

Thailand (latitude 5° 37' - 20° 27' N and longitude 97° 22' - 105°37' E) is located in Southeast Asia between Laos, Cambodia, Myanmar and Peninsular Malaysia and has an area of 513,900 km^2. The country comprises 76 provinces which are distributed in five regions, namely, North (14 provinces), Northeast (21

provinces), Central (21 provinces), East (6 provinces) and South (14 provinces). The fish collection localities are shown in Fig. 2.1 and information on the collection sites are summarized in Table 2.1. The sites were chosen after a literature search of areas where the catfish species of interest could be found. Not all the 98 recorded species of catfish in Thailand could be collected. The 32 localities are located in 17 provinces distributed in the five regions as follows: east, 1; central, 9; north, 6; northeast, 6; and south, 10.

The majority of fish species examined were wild, caught in rivers, reservoirs, torrential streams and swamps. Some of the fish species (*Hemibagrus nemurus*, *Pangasianodon hypophthalmus*, *P. gigas*, *Pangasius conchophilus*, *Clarias gariepinus* and *Clarias* hybrid) were obtained from culture systems. Fishes were also obtained from the ponds located in the Fishery Stations and Freshwater Fisheries Development Centers (FFDC) of the Department of Fisheries; Freshwater Fish Aquarium of the National Inland Fisheries Institute (NIFI), and in the agricultural campuses of Rajamangala Institute of Technology (RIT). Of the 32 fish collection localities, 20 localities could be classified as natural water systems (rivers, streams, reservoirs, swamps and torrential streams), while the others were man-made systems (Table 2.1). Brief descriptions of representative localities are given to provide a better understanding of the hosts' environment.

(a) Nan River in North Thailand (No. 6 in Fig. 2.1)

This 600-km long river originates in the Luang Prabang Ridge, Pua District, Nan, in the northern part of Thailand. The river runs through Nan, Uttaradit, Pitsanulok, Phichit and is joined by the Ping, Wang and Yhom rivers at Paknam Poh, Nakorn-sawan. There is a paucity of fish data from this river. Leenanond, Kittivorachate and Sricharoendham (1993) surveyed the section of this river before it joins with the other three rivers (in the Nakorn-sawan area) and recorded 62 fish species. The most common fishes are cyprinids. The catfishes present belong to the Siluridae (seven species), Pangasiidae (five species), Bagridae (five species) and Clariidae (one species). Gill net is the most popular gear used by fishermen in this area. Cage culture of *Channa micropeltes* was also widespread along this river (Leenanond *et al.*, 1993).

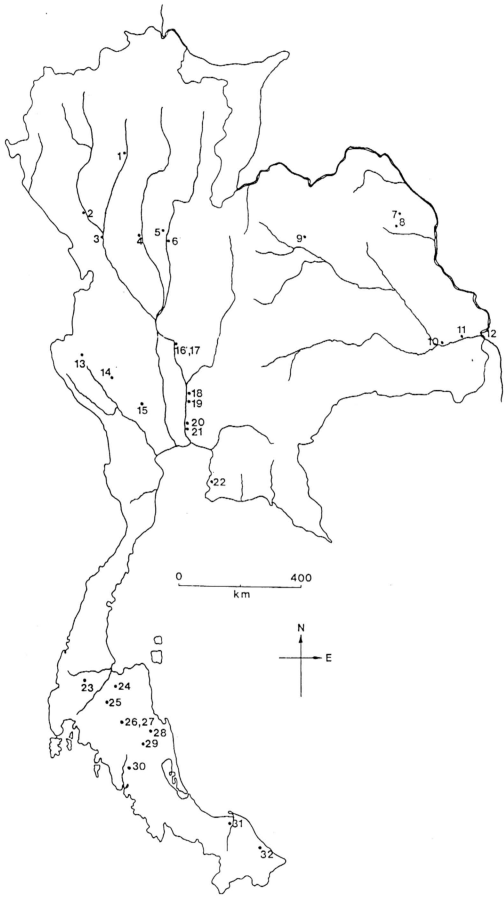

Fig. 2.1 Map of Thailand: Fish collection localities

Table 2.1 Collection sites of catfishes

Regions	Provinces	Sites	Remarks
1.North	1.Lumpang	1. Lampang Campus (RIT)	pond culture
	2.Tak	2. Bhumipol Reservoir	wild
		3. Ping River	wild
	3.Sukhothai	4. Yhom River	wild
	4.Pitsanulok	5. FFDC (Pitsanulok)	pond culture
		6. Nan River	wild
2.Northeast	5.Sakol-nakorn	7. FFDC (Sakol-nakorn)	pond culture
		8. Nong-han Swamp	wild
	6.Khon-kean	9. Ubonratana Reservoir	wild
	7.Ubonratchathanee	10. FFDC (Ubonratchathanee)	pond culture
		11. Mun River	wild
		12. Me-kong River	wild
3.Central	8.Kanchanaburi	13. Khao-Laem Reservoir	wild
		14. Srinakarin Reservoir	wild
		15. FFDC (Kanchanaburi)	net cage culture
	9.Chinat	16. Chao-praya River	wild
		17. FFDC (Chinat)	pond culture
	10.Ayudthaya	18. The Chao-praya River	wild
		19. Ayudthaya Campus (RIT)	pond culture
	11.Bangkok	20. NIFI Aquarium	aquarium fish
		21. Jatujak fish market	aquarium fish
4.East	12.Chonburi	22. FFDC (Chonburi)	pond culture
5.South	13.Surat-thani	23. Rajjaprabha Reservoir	wild
		34. FFDC (Surat-thani)	pond culture
		25. Tapi River	wild
	14.Nakornsithammarat	26. Nakornsithammarat Campus (RIT)	pond culture
		27. Yong Waterfall National Park	wild
		28. Khog-kram subdistrict	wild
		29. Cha-uad district	wild
	15.Trang	30. Trang River	wild
	16.Pattanee	31. Muang district	wild
	17.Narathiwas	32. Acid sulphate soil swamp	wild

(b) Nong Han Swamp, Sakol-nakorn in Northeast Thailand (No. 8 in Fig. 2.1)

Nong Han is the largest natural water resource in northeastern Thailand. It is located in Muang District, Sakol-nakorn and has a total area of 1,500 km^2. The Nong Han reservoir is fed by 15 rivers and streams. Nong Han is drained by the Kam river which runs through Nakhon Phanom to join the Me-kong River at the That-Phanom District in Nakorn-phanom. Sricharoendham and Koanantakul (1993) recorded 46 fish species belonging to 16 families in this reservoir. Cyprinidae constituted the largest group (18 species). The catfish species include *Clarias batrachus*, *Clarias macrocephalus*, *Kryptopterus bleekeri* and *Ompok bimaculatus*. Twelve types of fishing gears are used by fishermen, gill net being the most popular gear (used by 45 %).

(c) Mun River, Pak Mun Reservoir Area, Ubonratchathanee in Northeast Thailand (No. 12 in Fig. 2.1)

The Mun River is the longest river in the northeast region. The origin of this 641-km. long river is in the Ea-jan Ridge, Nakorn- ratchasima. This river runs through four provinces (Nakorn-ratchasima, Buri Ram, Surin and Sisaket) before joining Me-kong River at Pak Mun, Ubonratchathanee. Fifty-one fish species belonging to 19 families have been recorded from this river. The dominant fish group in terms of species is the cyprinids (31 %), murrels (20 %) and catfish (10 %) (Duangsawadi, Chookajorn, Karnasuta, Chantsavang, Leenanond & Sricharoendham, 1993). Purse seine is the most popular fishing gear used in this river. Other gears are gill net, long-line hooks, cast net, harpoon gun and bamboo trap (Duangsawadi *et al.*, 1993).

d) Khao-Laem Reservoir, Kanchanaburi in Central Thailand (No. 13 in Fig. 2.1)

The reservoir of Khao-Laem Dam, the fourth largest reservoir in Thailand, is on the Kuaew-Noi River. It was constructed for the generation of hydro-electric power and irrigation. The dam is located at Tongpapum District, Kanchanaburi. Sixty-six fish species were recorded from this reservoir. The dominant species belong to the families Cyprinidae (five species), Nandidae (one species), Bagridae (one species), Anabantidae (one species), Channidae (one species) and Notopteridae

(one species). Although the diversity of this reservoir is not high, its gross productivity is comparatively higher than other reservoirs (Nakjinda, Udomkananat, Tungkasen, Uttaranak & Prakobtham, 1986). Gill net is the most popular fishing gear in this area (59 %); other gears used include trap net (15 %), long-line hooks (12 %), while dip net, hook and line as well as spear make up 14 % (Sricharoendham, Leenanond, Kittivorachate, Kaewjaroon & Limbunjong, 1994).

(e) Srinakarin Reservoir, Kanchanaburi in Central Thailand (No. 14 in Fig. 2.1)

Srinakarin Reservoir in the Kuaew-Yai River, one of the largest reservoirs in Thailand, is located at the Sri-sawat District, Kanchanaburi. The reservoir has an area of about 400 km^2. Although the reservoir has provided a higher fish production, the dam has also resulted in a lower fish production in the waters below. Chantsavang, Ratanachumnong, Chaiboonthun, Kaewjaroon and Poomikong (1994) recorded 35 fish species from 17 families, viz., cyprinids (19.7 %), murrels (6.3 %), catfishes (2.6 %) and miscellaneous (71.3 %).

(f) Rajjaprabha Reservoir, Surat-thani in South Thailand (No. 23 in Fig. 2.1)

The Rajjaprabha Reservoir, with 176 km^2 of surface area, was formed by blocking the Klong Sang and seven minor canals at Ban Ta-khun District, Surat-thani. Prior to the dam construction, the eight canals joined the Tapi River (the main river in Surat-thani) which flows into the Gulf of Thailand at Ban Don Bay, Muang District, Surat-thani. In 1994, Duangsawasdi and Krachangdara collected 81 species of fish belonging to 21 families. Cyprinidae was dominant comprising 36 fish species. The other fish families recorded include the Amblycipitidae (one species) Anabantidae (four species), Bagridae (five species), Belonidae (one species), Centropomidae (one species), Channidae (five species), Clariidae (three species), Cobitidae (eight species), Eleotridae (one species), Gyrinocheilidae (one species), Homalopteridae (one species), Mastacembelidae (three species), Notop-teridae (two species), Osteoglossidae (one species), Pangasiidae (one species), Plistolepidae (one species), Siluridae (three species), Symbranchidae (one species), Syngnathtidae (one species) and Tetraodontidae (one species). A year later, in 1995, only 44 fish species belonging to 16 families were found. Of these 44 fish species, only four species are

siluriforms: *Hemibagrus nemurus* (Bagridae), *Batasio tengara* (Bagridae), *Clarias meladerma* (Clariidae) and *Ompok bimacu-latus* (Siluridae) (see Chantsavang, Ratanachumnong, Kaewjaroon and Poomikong, 1995).

Gill net is the common fishing gear used (37 %) in this area, followed by long-line hooks (19 %) and fish trap (13 %) (Sricharoendham, Leenanond and Poomikong, 1995).

(g) Freshwater swamp of Narathiwas in South Thailand (No. 32 in Fig. 2.1)

This freshwater swamp is located in a flat plain in the watershed area of Narathiwas province. It has a surface area of approximately 43,200 hectares (unpublished data). This swamp has acid soil composed of organic matters and pyrite (FeS_2) which produces sulfuric acid (H_2SO_4) by oxidation reaction resulting in acid water. Two clariid species, *Clarias teysmanni* and *C. nieuhofi* as well as *Chaca bankanensis* (Chacidae) were found in this area (Vidthayanon, pers.com.).

2.2.2 Methods of fish collection

The fishes were not collected according to any time or seasonal schedule because the purpose of this collection was simply to determine the monogeneans available on as many of the catfish species as possible because of the pioneering of this study (Section 1.5). Fish were caught using several methods including gill net, cast net, hook and line, long line baiting, fish trap and even electro-fishing method. Long line baiting is popular in big rivers in the northeastern region; while cast net, hook and line and fish traps are widely used in the watershed areas. Electro-fishing method was resorted only in standing waters such as small areas within reservoirs. However, due to difficulties in catching some catfish species, samples of these species were also bought alive from local fish markets.

The fishes collected were identified using the existing keys (Department of Fisheries, 1992; Duangsawadi & Krachangdara, 1994; Faculty of Fisheries, 1985; Kottelat, Whitten, Kartikasari, & Wirjoatmodjo, 1993; Mo, 1991; Mongkolprasit, Sonthiratana & Wongratana, 1980; Roberts, 1983, 1989, 1992a, 1994; Smith, 1945; Vidthayanon & Roongthongbaisuree, 1993) and confirmed by Dr. C. Vidthayanon, fish taxonomist of National Inland Fisheries Institute (NIFI), Bangkok, Thailand.

Table 2.2 Fish species examined and their localities

(C = Central, E = East, N = North, NE = Northeast, S = South)

(* potential cultured species)

Fish species	Localities	No. of Fish examined	No. of Fish infected
Ariidae:			
Hemipimelodus borneensis	Bangkok (C)	5	4
Bagridae:			
Bargrichthys macropterus	Ubonratchathanee (NE)	4	0
	Chinat (C)	1	0
Batasio tengara	Nakornsithammarat (S)	1	1
Hemibagrus nemurus *	Nakornsithammarat (S)	8	3
	Trang (S)	3	3
	Pattanee (S)	1	1
	Kanchanaburi (C)	10	9
	Ayuthaya (C)	1	0
	Chinat (C)	3	2
	Tak (N)	2	2
	Ubonratchathanee (NE)	11	10
	Khon-kean (NE)	3	3
Hemibagrus wyckii *	Ubonratchathanee (NE)	4	0
	Chinat (C)	1	0
	Kanchanaburi (C)	1	0
Hemibagrus wyckoides *	Surat-thani (S)	1	0
	Kanchanaburi (C)	1	1
	Tak (N)	1	1
	Khon-kean (NE)	1	1
	Ubonratchathanee (NE)	8	8
Mystus atrifasciatus	Ubonratchathanee (NE)	2	2
	Khon-kean (NE)	2	2
Mystus bocourti	Karnchanaburi (C)	1	1
	Tak (N)	1	1
	Chinat (C)	3	2

Table 2.2 cont'd

Fish species	Localities	No. of Fish examined	No. of Fish infected
Mystus gulio	Nakornsithammarat (S)	3	3
Mystus mysticetus	Bangkok (C)	1	1
Mystus singaringan	Nakornsithammarat (S)	4	3
	Pattanee (S)	1	1
	Chinat (C)	2	2
	Ubonratchathanee (NE)	1	1
	Khon-kean (NE)	1	1
Mystus wolffii	Nakornsithammarat (S)	4	4
	Suratthani (S)	1	1
	Chinat (C)	1	1
Pseudomystus siamensis	Nakornsithammarat (S)	9	8
Clariidae:			
Clarias batrachus *	Nakornsithammarat (S)	3	2
	Pattanee (S)	3	3
	Sakol-nakorn (NE)	1	1
	Ubonratchathanee (NE)	5	5
Clarias cataractus	Nakornsithammarat (S)	5	4
Clarias macrocephalus *	Nakornsithammarat (S)	8	3
	Pattanee (S)	1	1
	Sakol-nakorn (NE)	6	5
	Khon-kean (NE)	1	1
Clarias nieuhofi *	Nakornsithammarat (S)	9	6
	Narathiwas (S)	2	2
Clarias gariepinus *	Nakornsithammarat (S)	2	0
	Lumpang (N)	2	2
Clarias meladerma	Nakornsithammarat (S)	4	4
Clarias hybrid *	Nakornsithammarat (S)	10	8
	Pattanee (S)	2	0
	Ayudthaya (C)	3	2
	Lumpang (N)	10	4
	Ubonratchathanee (NE)	2	2

Table 2.2 cont'd

Fish species	Localities	No. of Fish examined	No. of Fish infected
Heteropneustidae:			
Heteropneustes fossilis *	Surat-thani (S)	10	7
Pangasiidae:			
Helicophagus waandersii	Ubonratchathanee (NE)	7	7
Pangasianodon gigas *	Surat-thani (S)	1	0
	Chinat (C)	2	0
	Pitsanulok (N)	1	0
	Sakol-nakorn (NE)	2	0
	Ubonratchathanee (NE)	1	0
*Pangasianodon hypophthalmus**	Nakornsithammarat (S)	1	1
	Bangkok (C)	2	2
	Ayudthaya (C)	2	2
	Pitsanulok (N)	2	2
	Tak (N)	1	1
	Ubonratchathanee (NE)	1	1
Pangsius bocourti *	Ubonratchathanee (NE)	8	7
Pangasius conchophilus *	Pitsanulok (N)	3	3
	Ubonratchathanee (NE)	5	5
	Chinat (C)	2	2
Pangasius krempfi	Ubonratchathanee (NE)	2	2
Pangasius larnaudii *	Ubonratchathanee (NE)	3	3
	Chinat (C)	1	1
Pangasius macronema	Tak (N)	7	7
	Chinat (C)	4	4
Pangasius sanitwongsei *	Bangkok (C)	2	2
Pteropangasius pleurotaenia	Ubonratchathanee (NE)	6	6
	Chinat (C)	1	1
Schilbeidae:			
Laides hexanema	Chinat (C)	3	3
	Tak (N)	1	1

Table 2.2 cont'd

Fish species	Localities	No. of Fish examined	No. of Fish infected
Siluridae:			
Belodontichthys dinema	Ubonratchathanee (NE)	4	4
Hemisilurus mekongensis	Ubonratchathanee (NE)	1	1
Kryptopterus apogon	Ubonratchathanee (NE)	4	2
	Chinat (C)	1	1
Kryptopterus bleekeri	Chinat (C)	2	2
	Khon-kean (NE)	3	3
	Sakol-nakorn (NE)	2	2
	Ubonratchathanee (NE)	2	1
Kryptopterus bicirrhis	Bangkok (C)	2	2
Kryptopterus cryptopterus	Bangkok (C)	2	2
	Ubonratchathanee (NE)	3	3
	Chinat (C)	1	1
Ompok bimaculatus *	Nakornsithammarat (S)	5	3
	Trang (S)	5	5
	Pattanee (S)	1	1
	Chonburi (C)	8	8
	Sukhothai (N)	1	1
	Khon-kean (NE)	1	1
	Sakol-nakorn (NE)	1	1
Silurichthys sp.	Nakornsithammrat (S)	3	2
Wallago attu	Ayudthaya (C)	1	1
	Tak (N)	1	1
	Sukhothai (N)	1	1
	Pitsanulok (N)	1	1
	Chinat (C)	1	1
Sisoridae:			
Bagarius bagarius	Tak (N)	2	1
	Ubonratchathanee (NE)	3	1
	Chinat (C)	1	0
Bagarius yarrelli	Chinat (C)	2	0
Glyptothorax major	Nakornsithammarat	2	2

As far as possible each catfish species was photographed (Appendix 2) and specimens of the host species were kept at the Nakornsithammarat Campus, Rajamangala Institute of Technology (RIT) for future reference. This is done because there are still some taxonomic questions regarding some of the fish species.

A total of 335 fish specimens belonging to 44 catfish species, 21 genera and eight families were examined in this study. The number of fishes examined and the number of fish infected by monogeneans are given in Table 2.2.

2.3 Monogenean collection

2.3.1 Collection, preparation and preservation of monogeneans

The fish specimens were killed by severing the spinal cord with a sharp blade or by pitting the brain. Measurements of the fish were taken and recorded. The fishes were preserved for taxonomic references and kept at the Nakornsi-thammarat Campus of Rajamangala Institute of Technology for future references. The gills were removed and placed in petri dishes containing clean local water. The fresh gills were gently scraped with a bent needle to dislodge the monogeneans. The dislodged monogeneans were picked out under the dissecting microscope using a small fine pipette. The monogeneans were then dropped onto a clean glass slide with a small drop of water. A cover slip was gently dropped onto the monogenean specimens.

Excess water on the slides were dried off and the four corners of the coverslip were sealed with nail varnish to prevent the coverslip from shifting. Ammonium-picrate-glycerine (modified from Malmberg's formula) (Malmberg, 1970; Lim, 1991a) was added to the edge of the coverslip and allowed to drain beneath the coverslip to fix and clear the specimens. Excess fixative was removed and the sides of coverslip were sealed with nail varnish. The monogeneans were then examined under the phase contrast microscope. Some live specimens were examined for soft part anatomy. To make permanent mounts, the coverslip was removed and the specimens on the slide were dehydrated in graded alcohol series, clear in Xylene and mounted in Canada balsam (see Lim, 1991a: Ergens' method).

2.3.2 Identification and descriptions of monogeneans

The monogeneans collected were identified and the new species described. Although the identifications and descriptions of the monogeneans are the focal point of this project (especially since 66 of the 83 species are new) the descriptions are given in the Appendices 3.1-3.8. This is to facilitate the analysis of the host-monogenean data as well as the morphological data and to make the thesis less tedious to read.

The monogeneans were mainly identified on the basis of hard sclerotised parts (haptoral armaments, copulatory organs and vaginal armaments). The hard parts were measured in micrometers (μm.) as outlined by Lim (1986a, 1991b, 1996a). The terminologies used are those of Gussev (1976) and Lim (1991b). However as far as possible the descriptions of the soft parts are also given (see Appendices 3.1-3.8). The monogeneans collected were compared with previously described species from the Indian subcontinent, Peninsular Malaysia, Indo-China, South China, the Palaearctic and Amur-Chinese regions as well as the Ethiopian region (see Appendix 1).

2.4 Analyses of data

2.4.1 Distribution data: prevalence and mean intensity

The number of fish specimens collected and examined (infected and non-infected) from the 32 localities as well as number of monogeneans collected were recorded (Section 4.2; Table 4.1) and the prevalence and mean intensity (as defined by Margolis, Esch, Holmes, Kuris & Schad, 1982) were calculated (Table 4.1).

$$\text{Prevalence (\%)} = \frac{\text{No. of fish infected with a particular monogenean species}}{\text{No. of fish examined}} \times 100$$

$$\text{Mean intensity} = \frac{\text{Total No. of individuals of a particular monogenean species}}{\text{No. of fish infected by that particular monogenean species}}$$

2.4.2 Morphological analysis

The aims of this section are to determine if it was possible to use the morphology of the monogeneans to group the different host species (Chapter 5 discusses the rationale) and to determine the morphological diversity of the

monogeneans. Briefly, the methods employed include categorising into traits the different sclerotised parts of the monogenean species obtained providing codes for each of the traits, and characterising the different monogenean species obtained using these codes. The results are tabulated in Tables 5.11 & 5.12 (Section 5.2). The similarities between these monogeneans were calculated (see below), and resulting data summarised using the nearest neighbor sorting method in the Cluster Analysis programme found in the Statistical Analysis System package (SAS) to determine the relationships between monogenean species (under the Ward's method) (SAS/STAT Guide for Personal Computers, Version 6 Edition, SAS Institute Inc., 1987). The results of cluster analysis were summarised in dendrograms (Figs. 5.1 - 5.20; Section 5.3).

2.4.2.1 Categorisation (coding) of sclerotised structures

The traits of the different sclerotised parts are categorised and codes are given for each categories (Figs. 2.2 - 2.13).

(i) Sclerotised haptoral parts

(1) Anchors (Figs. 2.2 & 2.3; Tables 2.3 & 2.4)

Anchors are the main structures for attachment of the monogeneans. The ancylodiscoidins and ancyrocephalins have two pairs of anchors (dorsal and ventral). Eleven types of dorsal anchors (Fig. 2.2 & Table 2.3) and 15 types of ventral anchors (Fig. 2.3 & Table 2.4) could be categorised from the 83 monogenean species in the present study. The dorsal anchors of these 83 species vary from small anchors without root (DA1) to disproportionately large anchors (DA7). Of the 11 types of dorsal anchors, seven types were categorised from ancylodiscoidins, while the other four types were observed in ancyrocephalins. Twelve types of ventral anchors were found in the ancylodiscoidin species, while four types are found in the ancyrocephalins with three types being unique to the ancyrocephalins (VA5, VA6 & VA15).

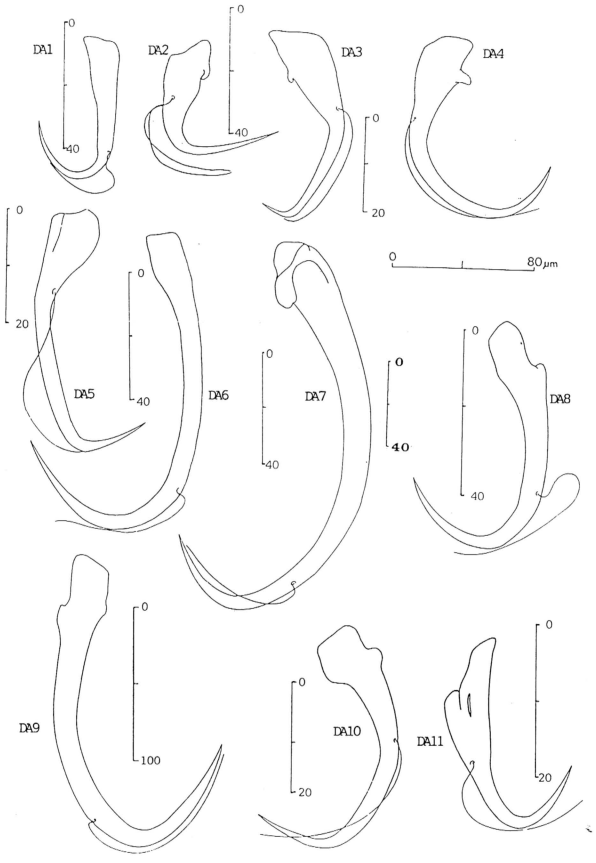

Fig. 2.2 Codes of different dorsal anchor types
(Scale bar in micrometer)

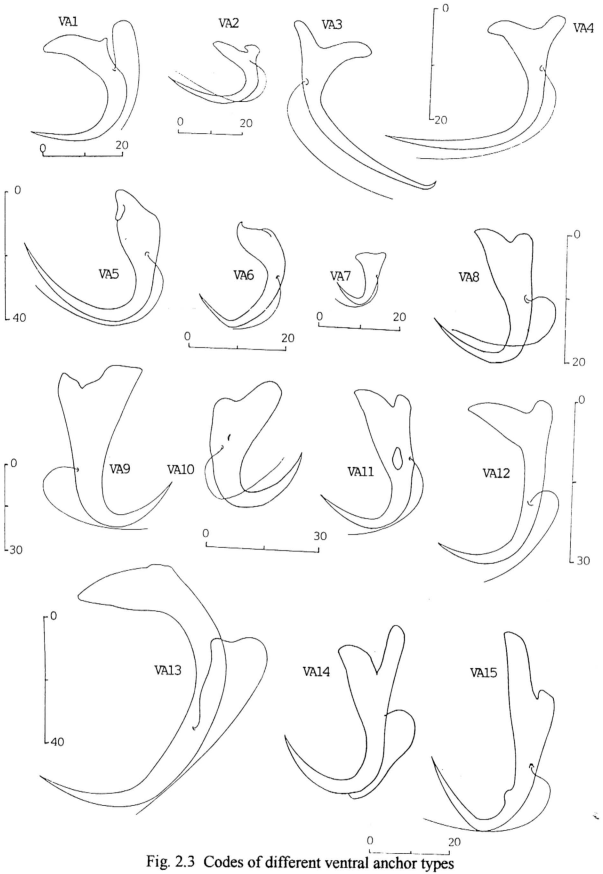

Fig. 2.3 Codes of different ventral anchor types

(Scale bar in micrometer)

Table 2.3 The characteristics of dorsal anchors of monogeneans from Thai freshwater siluriforms

Characteristics	Codes	Examples
1. Small size, without root	DA1	*Thaparocleidus* sp.27
2. *Quadriacanthus*-type	DA2	*Quadriacanthus* sp.1
3. *Bychowskyella*-type	DA3	*Bychowskyella* sp.4
4. *Mizelleus*-type	DA4	*Mizelleus* sp.1
5. Without root, long and slender shaft	DA5	*Thaparocleidus* sp.2
6. Large anchor, without root, slender shaft	DA6	*Cornudiscoides* sp.8
7. *Bifurcohaptor*-type	DA7	*Bifurcohaptor* sp. 2
8. Inner root well-developed, stumpy outer root	DA8	*Thaparocleidus* sp.17
9. Very large and masssive, inner root well-developed	DA9	*Thaparocleidus* sp.21
10. inner root club-shaped	DA10	*Thaparocleidus* sp.31
11. *Hamatopeduncularia*-type (roots well-developed)	DA11	*Hamatopeduncularla* sp.2

Table 2.4 The characteristics of ventral anchors of monogeneans from Thai freshwater siluriforms

Characteristics	Codes	Examples
1. Massive inner root, long recurved point	VA1	*Cornudiscoides* sp.1
2. Roots well-developed, gently recurved point	VA2	*Cornudiscoides* sp.8
3. Small anchors, long straight point with recurved tip	VA3	*Cornudiscoides* sp.10
4. Small anchor with long open point	VA4	*Cornudiscoides* sp.6
5. *Quadriacanthus*-type	VA5	*Quadriacanthus* sp.1
6. *Bychowskyella*-type	VA6	*Bychowskyella* sp.4
7. Stumpy anchor, without root, short recurved point	VA7	*Thaparocleidus* sp.46
8. Common shape; inner and outer root well-developed	VA8	*Thaparocleidus* sp.26
9. Massive, outer root well-developed	VA9	*Thaparocleidus* sp.25
10. Massive, fenestrated at mainpart, without root	VA10	*Thaparocleidus* sp.55
11. Common shape, fenestrated at mainpart	VA11	*Thaparocleidus* sp.32
12. Large, long straight shaft, function like forceps	VA12	*Thaparocleidus* sp.34
13. Large and massive, without root	VA13	*Thaparocleidus* sp.27
14. Outer root noticeably longer than inner root	VA14	*Thaparocleidus* sp.53
15. *Hamatopeduncularia*-type	VA15	*Hamatopeduncularia* sp.1

(2) Connective bars (Figs. 2.4 & 2.5; Tables 2.5 & 2.6)

Ancylodiscoidins and ancyrocephalins usually have two connective bars, dorsal and ventral. The dorsal bar articulates with the dorsal anchors, while the ventral bar articulates with the ventral anchors.

Eleven types of dorsal bar (Fig. 2.4 & Table 2.5) and 11 types of ventral bars (Fig. 2.5 & Table 2.6) were categorised from the 83 monogenean species obtained. Six types of dorsal bars and seven types of ventral bars were observed in the ancylodiscoidins, while four types of dorsal bars and four types of ventral bars were observed in the ancyrocephalins (Figs. 2.4 & 2.5).

Ventral bars may be paired or unpaired. Six types of paired ventral bars and five types of unpaired ventral bars were observed in this study (Figs. 2.4 & Table 2.6).

(3) Patches (Fig. 2.6 & Table 2.7)

Patches are observed on dorsal and ventral anchors, although they are normally located above the inner root of dorsal anchors of the ancylodiscoidins and ancyrocephalins. Patches located on the dorsal anchors are designated as dorsal patches, and on the ventral anchors as ventral patches.

In this study 11 types of dorsal patches and two types of ventral patches were categorised (Fig. 2.6 & Table 2.7). Nine types of dorsal patches are observed in ancylodiscoidins, while three types are observed in ancyrocephalins.

(4) Marginal hooks (Fig. 2.7 & Table 2.8)

The marginal hooks can be differentiated into larval and adult forms. In some monogeneans all the seven pairs of marginal hooks are of one form, while in some monogeneans the marginal hooks are made up of a combination of adult and larval forms. The shapes and sizes of the marginal hooks of the present monogeneans are variable. If all these characteristics were used too many traits would have been generated, creating too many artificial groupings. Therefore instead of categorising each marginal hooks, different combinations of the 7 pairs of marginal hooks are considered resulting in five types of combinations of marginal hooks (Figs. 2.7 & Table 2.8):

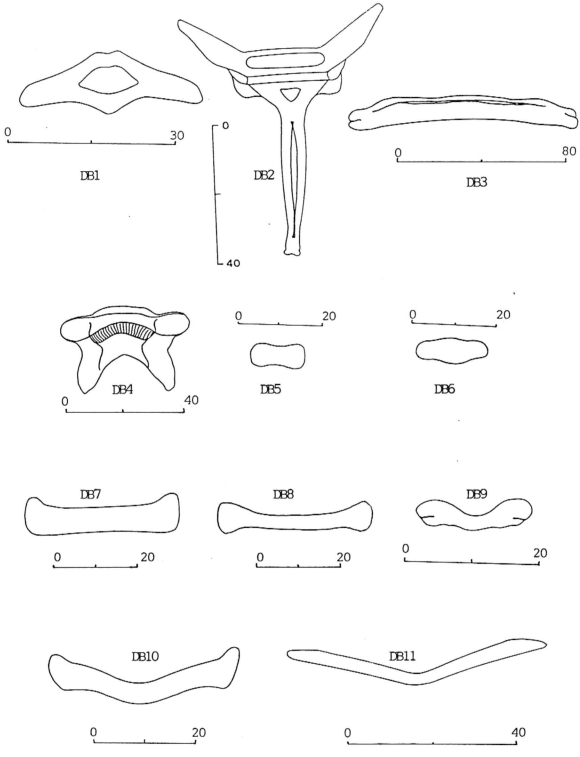

Fig. 2.4 Codes of different dorsal bar types
(Scale bar in micrometer)

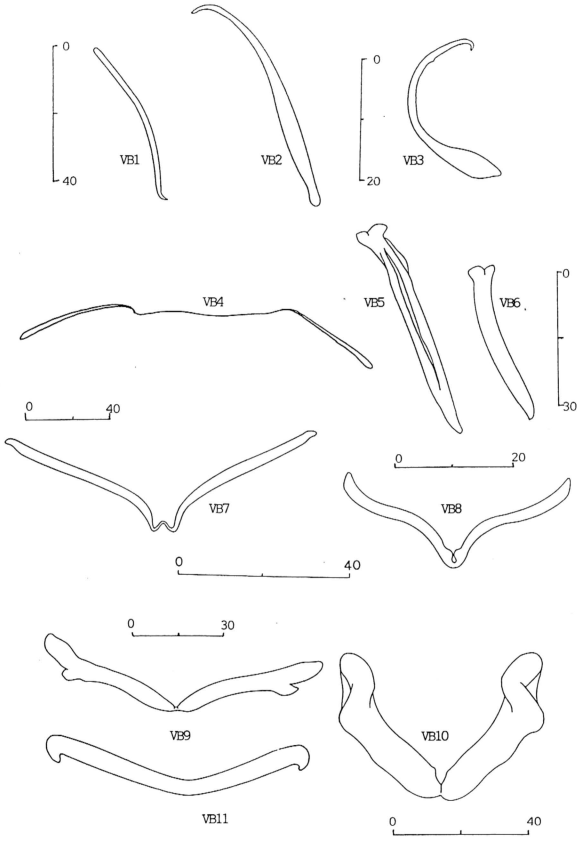

Fig. 2.5 Codes of different ventral bar types
(Scale bar in micrometer)

Table 2.5 The characteristics of dorsal bars of monogeneans from Thai freshwater siluriforms

Characteristics	Codes	Examples
1.Fenestrated in middle	DB1	*Bychowskyella* sp.4
2.T-shaped	DB2	*Quadriacanthus* sp.1
3.Fenestrated bar, *Mizelleus*-type	DB3	*Mizelleus* sp.1
4.Solid squared, ventral concaved	DB4	*Bifurcohaptor* sp.2
5.Small, dumbbell-shaped	DB5	*Cornudiscoides* sp.8
6.Small, spindle shaped	DB6	*Cornudiscoides* sp.11
7.Straight shaped	DB7	*Thaparocleidus* sp.28
8.Straight, bone-shaped	DB8	*Cornudiscoides* sp.1
9.Massive, broad V-shaped	DB9	*Thaparocleidus* sp. 9
10.Slightly V-shaped	DB10	*Thaparocleidus* sp. 8
11.Long V-shaped	DB11	*Hamatopeduncularia* sp.1

Table 2.6 The characteristics of ventral bars of monogeneans from Thai freshwater siluriforms

Characteristics	Codes	Examples
1.Long, slightly curved paired	VB1	*Bifurcohaptor* sp.2
2.Paired, without main part	VB2	*Cornudiscoides* sp.10
3.Paired with massive mainpart	VB3	*Cornudiscoides* sp.9
4.Paired, connected with ligament	VB4	*Cornudiscoides* sp.5
5.Paired, fenestrated, widening at proximal end	VB5	*Bychowskyella* sp. 4
6.Paired, non-fenestrated, widening at proximal end	VB6	*Bychowskyella* sp. 7
7.M-shaped, narrowing at middle	VB7	*Mizelleus* sp.1
8.Common V-shaped	VB8	*Thaparocleidus* sp.32
9.Massive V-shaped with protuberances at ventral side	VB9	*Thaparocleidus* sp.25
10.Large, massive V-shaped	VB10	*Thaparocleidus* sp.27
11.Broad V-shaped	VB11	*Hamatopeduncularia* sp.1

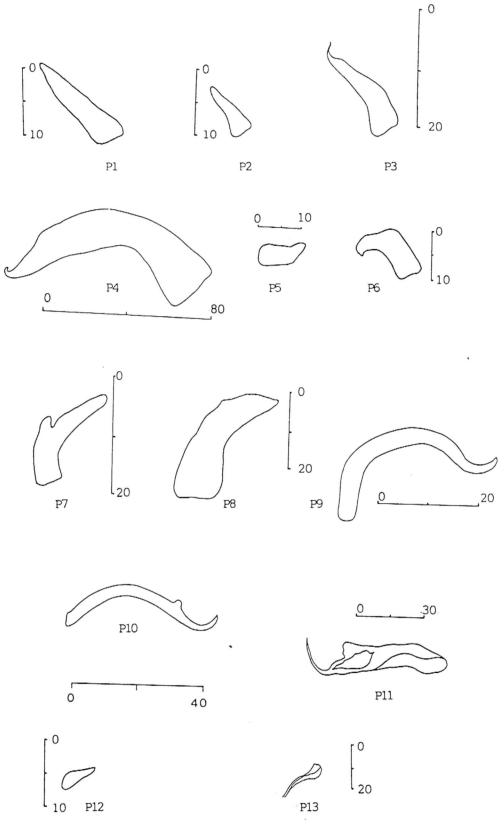

Fig. 2.6 Codes of different patch types

(Scale bar in micrometer)

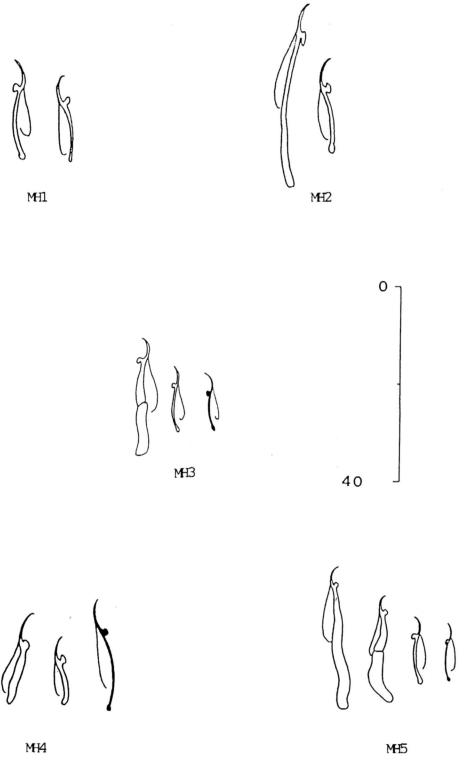

Fig. 2.7 Codes of different marginal hook forms
(Scale bar in micrometer)

1. Group I (MH1): where all the seven pairs are larval forms (Fig. 2.7)
2. Group II (MH2): where six pairs are larval forms and one pair of modified larval hooks (Fig. 2.7).
3. Group III (MH3): a combination of different forms of larval hooks (Fig. 2.7).
4. Group IV (MH4): two pairs of adult-type marginal hooks (medium size) with demarcated handle and five pairs larval hooks (Fig. 2.7).
5. Group V (MH5): *Bychowskyella*-type: large adult hooks with well-demarcated handle and larval hooks (Fig. 2.7).

(5) Sclerotised rods (Fig. 2.8)

Sclerotised rods near the dorsal anchors are present only in ancyrocephalins, *Bychowskyella* (see Fig. 2.8 & A40, for example).

(6) Onchium (Fig. 2.9)

Onchium is the extra sclerotised piece observed only in the haptors of the ancyrocephalins and not in the ancylodiscoidins. The number of onchia vary from zero to two.

(ii) Sclerotised reproductive parts

(1) Copulatory organs (Figs. 2.10 & 2.11; Tables 2.9 & 2.10)

The male copulatory organ of the monogeneans usually consists of a copulatory tube and accessory piece. Ten types of copulatory tubes were categorised (Fig. 2.10 & Table 2.9) ranging from simple tube (CT1) to complex coiled tubes (CT10). Ten types of accessory pieces were observed in monogeneans from the Thai catfish (Figs. 2.11 & Table 2.10). All the ten types of copulatory tubes are observed in the Ancylodiscoidinae (especially *Thaparocleidus*), while only two types (CT2 & CT7) are found in the Ancyrocephalinae. There are three types of coiled copulatory tube: (i) 2-5 coils (CT8), (ii) loosely coiled tube (CT9), and (iii) highly coiled tube (6-10 coils: CT10).

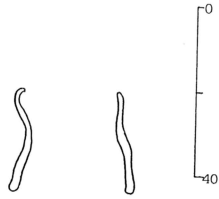

Fig. 2.8 Sclerotised rod of *Bychowskyella* species
(Sclae bar in micrometer)

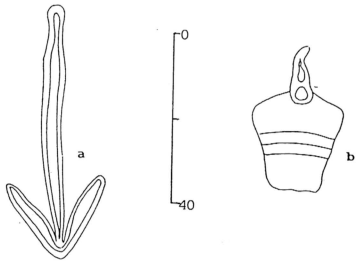

Fig. 2.9 Onchia of *Bychowskyella* species from sisorid fish
a: ventral onchium; b: dorsal onchium
(Scale bar in micrometer)

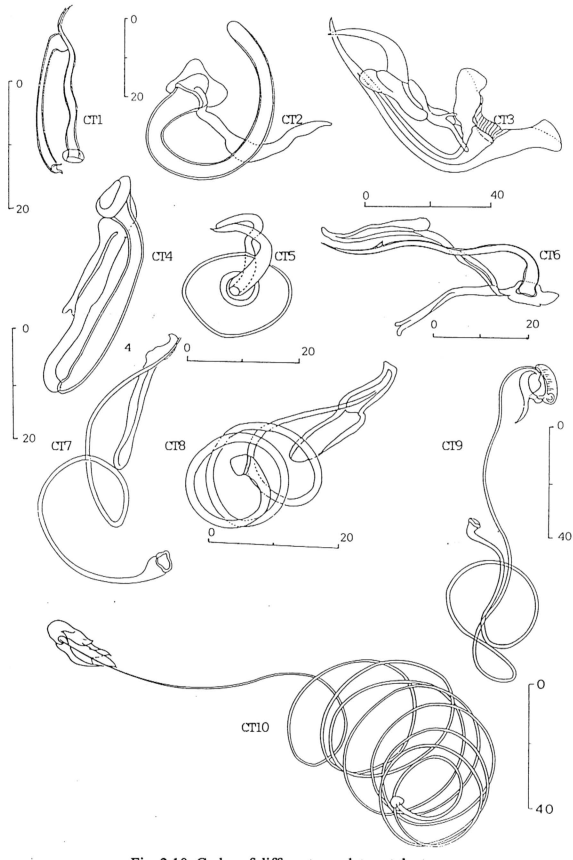

Fig. 2.10 Codes of different copulatory tube types

(Scale bar in micrometer)

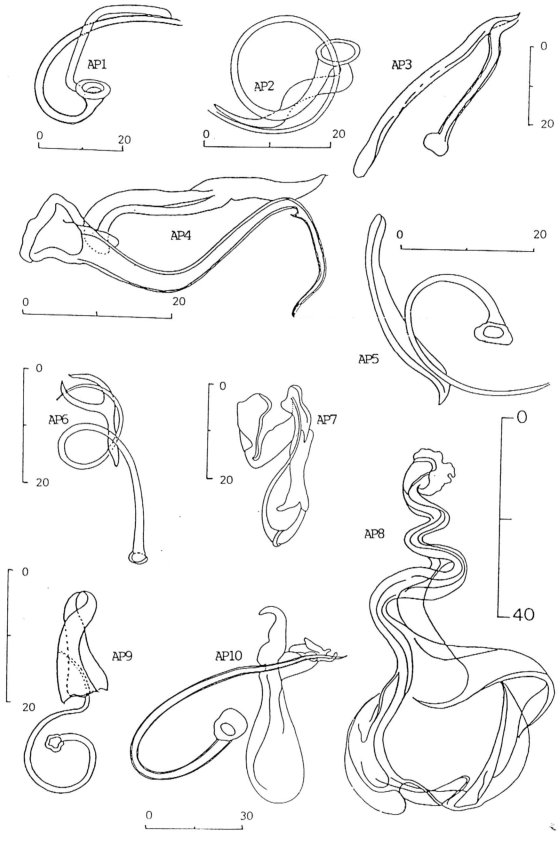

Fig. 2.11 Codes of different accessory piece types
(Scale bar in micrometer)

Table 2.9 The characteristics of copulatory tubes of monogeneans from Thai freshwater siluriforms

Characteristics	Codes	Examples
1.undulated tube	CT1	*Cornudiscoides* sp.9
2.Short curved tube	CT2	*Thaparocleidus* sp.28
3.Curved tube, expanded at initial part	CT3	*Thaparocleidus* sp.30
4.Sac-like	CT4	*Thaparocleidus* sp.32
5.Curved tube, ininitial part bulbous	CT5	*Thaparocleidus* sp.33
6.Sigmoid tube with spine near the distal part	CT6	*Thaparocleidus* sp.16
7.Long twisted curved tube	CT7	*Thaparocleidus* sp.2
8.Less coiled tube (2-5 coils)	CT8	*Thaparocleidus* sp.5
9.Very long, loosely coiled tube	CT9	*Bifurcohaptor* sp.2
10.Highly coiled tube (6-10 coils)	CT10	*Thaparocleidus* sp.38

Table 2.10 The characteristics of accessory pieces of monogeneans from Thai freshwater siluriforms

Characteristics	Codes	Examples
1.C-shaped rod	AP1	*Thaparocleidus* sp.17
2.sinuous rod-like	AP2	*Thaparocleidus* sp.15
3.Grooved or branched stick-like	AP3	*Bychowskyella* sp.6
4.Grooved sigmoid, stick-like	AP4	*Thaparocleidus* sp. 16
5.Grooved plate-like	AP5	*Cornudiscoides* sp.4
6.Forked stick-like	AP6	*Cornudiscoides* sp.3
7.Grooved piece with recurved shield	AP7	*Bychowskyella* sp.4
8.Undulated sac-like	AP8	*Thaparocleidus* sp.19
9.Clipper-like	AP9	*Thaparocleidus* sp.11
10.Bulbous	AP10	*Thaparocleidus* sp.24
11.No accessory piece	AP0	*Hamatopeduncularia* sp.1

(2) Vaginal armaments (Figs. 2.12 & 2.13, Tables 2.11 & 2.12)

Vaginal system is composed of sclerotised vaginal opening and a vaginal tube. Seven types of sclerotised vaginal opening were categorised (Fig. 2.12 & Table 2.11). Sclerotised vaginal opening was not observed in 18 monogenean species; *Bychowskyella* (three species), *Cornudiscoides* (two species), *Quadri-acanthus* (one species) and *Thaparocleidus* (12 species).

Seven types of vaginal tubes were observed (Figs. 2.13 & Table 2.12). Vaginal tubes were not observed in 21 species because the tubes are either lightly sclerotised or unsclerotised. The vaginal tubes vary from short straight tube (VT1), long curved tubes (VT5) to complex coiled tubes (VT7).

2.4.2.2 Non-sclerotised reproductive parts: seminal vesicles

The non-sclerotised reproductive parts are not used in the morphological analyses but is used in Chapter 6. Three types of seminal vesicles could be found in the monogeneans from freshwater catfishes: (i) single seminal vesicle of the dactylogyrid-type, (ii) two seminal vesicles of the dactylogyrid-type and (iii) single seminal vesicle of the blind-sac type. The *Anchylodiscus* species as exemplified by *Anchylodiscus liewi* Lim, 1992 possesses two seminal vesicles: a dactylogyrid-type and a blind-sac type (see Lim, 1992c).

There are three types of blind-sac like seminal vesicle based on the shape of the blind-sac: (a) long and thin, (b) pyriform and (c) ovoid. However, the morphology of the seminal vesicle is not included in the cluster analysis in this study. This is because of the paucity of information on the shapes of seminal vesicles of the different species of ancylodiscoidins.

2.4.2.3 Cluster analysis

Cluster analysis is a technique for combining obsevations into groups or clusters that are as homogeneous as possible with respect to the clustering variables or characterisitics (Sharma, 1996). In this study, the Cluster Analysis in the Statistical Analysis System (SAS) programme (Release 6.0. SAS, 1990) (SAS/STAT Guide for Personal Computers, Version 6 Edition. 1987. SAS Institute Inc. Cary, NC, 1987. 1028 p.) was used (i) to calculate the similarities between the characterised monogeneans, (ii) to summarise the resulting similarity measures

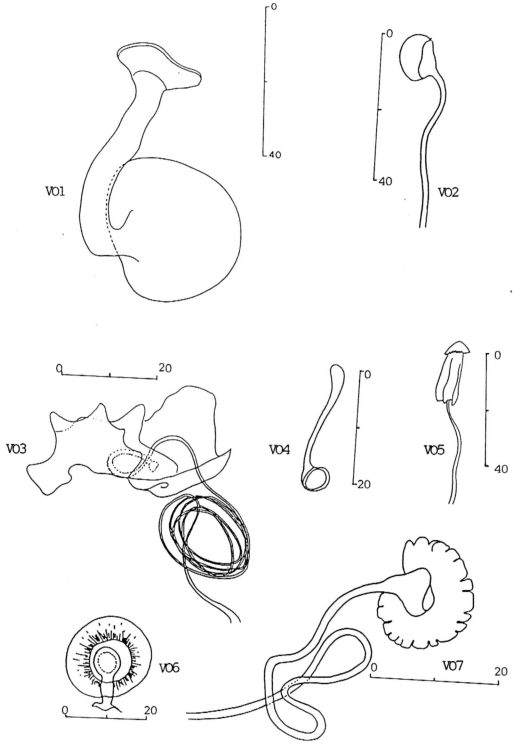

Fig. 2.12 Codes of different vaginal opening types

(scale bars in micrometer)

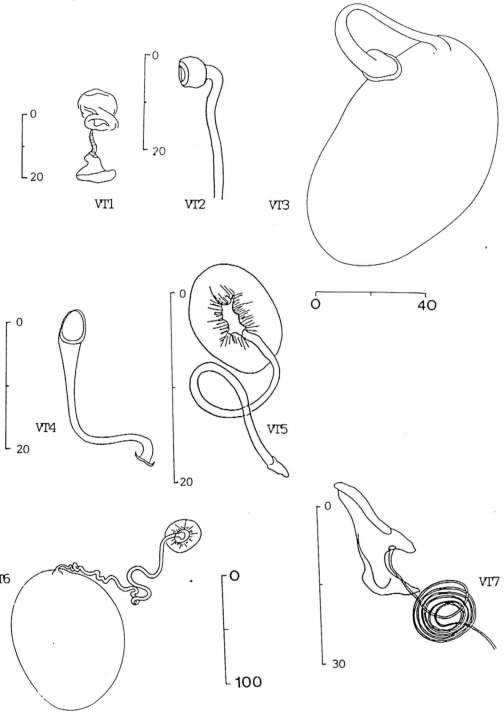

Fig. 2.13 Codes of different vaginal tube types

(scale bars in micrometer)

Table 2.11 The characteristics of vaginal openings of monogeneans from Thai freshwater siluriforms

Characteristics	Codes	Examples
1.Funnel-like	V01	*Thaparocleidus* sp.30
2.Globulate	V02	*Thaparocleidus* sp.8
3.Guarded with sclerotised piece	V03	*Thaparocleidus* sp.38
4.Finger-like	V04	*Cornudiscoides* sp.1
5.Arrow-shaped	V05	*Thaparocleidus* sp.24
6.Guarded with muscle	V06	*Thaparocleidus* sp.54
7.Flower-like	V07	*Thaparocleidus* sp.52
8.Not observed	V00	*Thaparocleidus* sp.3

Table 2.12 The characteristics of vaginal tubes of monogeneans from Thai freshwater siluriforms

Characteristics	Codes	Examples
1.Short straight tube	VT1	*Quadriacanthus* sp.1
2.Long straight tube	VT2	*Thaparocleidus* sp.5
3.Short curved tube	VT3	*Thaparocleidus* sp.21
4.Sigmoid tube	VT4	*Thaparocleidus* sp.29
5.Long curved tube	VT5	*Thaparocleidus* sp.54
6.Loosely, irregular coiled tube	VT6	*Thaparocleidus* sp.25
7.Highly coiled tube	VT7	*Thaparocleidus* sp.35
8.Not observed	VT0	*Thaparocleidus* sp.55

using a clustering technique and (iii) clusters evaluated using the semipartial R-squared statistics (Sharma, 1996).

In this study the similarities between the monogeneans are measured by the squared euclidean distances (see Sharma, 1996 Chapter 7), while Ward's hierarchical clustering method is used to cluster the resulting indices of similarities based on euclidean distances. Ward's method in hierarchical clustering was used as the clustering technique in this analysis to form clusters by maximising within-cluster homogeneity. Ward's hierarchical clustering is used because it generates clusters, which are more uniform in size than the other methods such as, for example, the centroid method and average linkage method (Griffith & Amrhein, 1997).

The outputs generated are summarised in the form of dendrogram using the hierarchical clustering programme procedure in SAS (PROC CLUSTER) (Sharma, 1996 Chapter 7). The clusters generated could be evaluated by a number of statistics: in this study the semipartial R-squared (SPR) was chosen. The details for **this statistic are found in Sharma (1996). Briefly, SPR is the loss of homogeneity** obtained when two groups or clusters are joined together to form a new cluster. SPR is obtained by dividing the difference between the pooled SS (or standard deviations) of the new cluster and the sum of the pooled SS of the clusters joined to form the new cluster by the pooled SS for the total sample. A smaller SPR value (or small loss of homogeneity) implies that two homogenous groups were merged together while a larger value suggests that the two heterogeneous groups were merged.

Several dendrograms of relationships were generated for all the ancylodiscoidins, all the ancyrocephalins as well as for all the species within *Thaparocleidus* and *Cornudiscoides*. The 20 **dendrograms** generated are based on the use of all the sclerotised parts, the haptoral armaments and reproductive structures (Figs. 5.1 - 5.20; Section 5.3). The monogenean relationships as noted in the **dendrograms** were correlated with the fish hosts and discussed in Chapter 5.

2.5 Limitations and delimitations of study

The study had the following limitations:

a. The number of catfish species collected and examined were variable, because of the difficulty in obtaining some species which occur in low numbers. Therefore only 44 species (or 45 %) of the 98 known catfish species were examined.

b. Although monogeneans have been reported from the nasal cavity of clariids (see Ergens, 1988; Kritsky & Kulo, 1988), only gills were examined due to time constraint.

c. No data on monthly distribution patterns of monogeneans were collected due to the time and manpower constraints.

d. Due to the variable number of catfish species examined, the monogenean diversity indic cs could not be calculated.

e. Although hard parts were used to determine monogenean relationships, the morphometrical differences were not considered.

f. The SAS cluster analysis method was used for the morphological analysis because this programme was easily available.