COMPARATIVE TAXONOMY AND BREEDING BEHAVIOUR OF FIVE POPULATIONS OF *Betta pugnax* GROUP (OSPHRONEMIDAE) FROM JOHOR, MALAYSIA

by

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PERBANDINGAN TAKSONOMI DAN KELAKUAN PEMBIAKAN LIMA POPULASI KUMPULAN *Betta pugnax* (OSPHRONEMIDAE) DARI JOHOR, MALAYSIA

ABSTRAK

Taksonomi dan kelakuan pembiakan kumpulan *Betta pugnax* berdasarkan lima populasi dari Johor, Malaysia telah dikaji. Teknik meristik, morfometrik tradisional dan morfometrik jaringan truss telah dijalankan ke atas lima populasi ini untuk menilai status taksonomi dan pembezaan morfologi kuantitatif. Disamping itu, warna dan habitat telah direkod. Data kuantitatif telah dianalisis melalui analisis univariat varians (ANOVA) dan analisis multivariat iaitu analisis komponen utama (PCA) dan analisis fungsi diskriminan (DFA). Tiga populasi dari Hutan Simpanan Kekal Mersing (I), Kampung Sinar Harapan (III) dan Kampung Parit Jepon (IV) telah dicamkan sebagai *B. pugnax*. Terdapat variasi yang signifikan bagi kedua-dua pendekatan morfometrik antara ketiga-tiga populasi ini. Tetapi tiada perbezaan yang signifikan pada data meristik telah diperhatikan. Ketiga-tiga populasi berkongsi warna dan kelakuan pembiakan yang sama. Mereka dijumpai hidup di perairan jernih and juga perairan hitam yang belum didokumentasi sebelum ini. Populasi dari Parit Lapis 2 (V) dicamkan sebagai *B. pulchra*. Spesies ini boleh dibezakan daripada *B. pugnax* dengan mempunyai badan yang lebih lebar. Bentuk sirip kaudal dan intensiti sisik iridesen *B. pulchra* adalah berubah-ubah dan tidak boleh dijadikan kekunci pengecaman spesies ini. *Betta pulchra* mempunyai kelakuan pembiakan yang sama dengan *B. pugnax*. Populasi (II) yang disampel bersama dengan *B. pugnax* di Hutan Simpanan Kekal Mersing tidak meyerupai mana-mana spesies

daripada kumpulan *B. pugnax* dan berkemungkinan besar merupakan spesies yang baru. Populasi ini boleh dibeza dengan spesies lain daripada kumpulan *B. pugnax* dengan mempunyai kombinasi sifat unik yang berikutnya: tinggi badan 21.9-26.7% panjang piawai; panjang kepala 29.1-32.2% panjang piawai; sisik garis deria 31-32; sisik melintang tubuh 8-8½; dagu berjalur; terdapat jalur pascaorbital yang kedua pada operkulum; warna biru yang tipis di tepi sirip dubur dan bahagian bawah sirip kaudal. Populasi ini juga mempunyai warna nuptial dan postur pemindahan telur yang berbeza dengan *B. pugnax* dan *B. pulchra*. Ikan betina populasi ini menunjuk postur yang oblik apabila bertentangan penceroboh di tempat pembiakannya berbanding dengan postur horizontal pada *B. pugnax* dan *B. pulchra*. Kesimpulan ini dikukuhkan lagi dengan ketidakupayaan populasi ini untuk membiak dengan spesies *B. pugnax*.

COMPARATIVE TAXONOMY AND BREEDING BEHAVIOUR OF FIVE POPULATIONS OF *Betta pugnax* GROUP (OSPHRONEMIDAE) FROM JOHOR, MALAYSIA

ABSTRACT

The taxonomy and breeding behaviour of the *Betta pugnax* group based on five populations from Johor, Malaysia was investigated. Meristic, traditional and truss network morphometric techniques were conducted on these populations to assess their taxonomy status and quantitative morphological differentiation. In addition, their colouration and habitat were also recorded. The quantitative data was analysed by univariate analysis of variance (ANOVA) and multivariate analyses of principal component analysis (PCA) and discriminant function analysis (DFA). The Mersing Forest Reserve (I), Kampung Sinar Harapan (III) and Kampung Parit Jepon (IV) populations were identified as *B. pugnax*. There were significant variations for both morphometric approaches among the three populations. The meristic counts showed no significant differences among all populations. Their colouration and reproductive behaviour were similar. They were found in both clearwater as well as blackwater habitats and this had not been previously reported. The population (V) from Parit Lapis 2 was identified as *B. pulchra*. It differed from *B. pugnax* by its stouter body. The caudal fin shape and the intensity of the iridescent scales of *B. pulchra* were changeable and unreliable for taxonomic identification. The reproductive behaviour of *B. pulchra* was similar to *B. pugnax.* The population (II) which was caught together with *B. pugnax* from the Mersing Forest Reserve did not resemble any member of the *B. pugnax* group previously documented and most

probably represented a new species. It differed from other members of the *B. pugnax* group in having the following unique combination of characters: body depth 21.9-26.7% of SL; head length 29.1-32.2% of SL; lateral scales 31-32; transversal scales 8-8½; having chin bar; second postorbital stripe on opercle; thin blue margin on anal fin and lower half of caudal fin. Its nuptial colouration and egg transferring posture were different from *B. pugnax* and *B. pulchra*. The female displayed an oblique posture when confronted by an intruder at the spawning site as compared to the horizontal position displayed by *B. pugnax* and *B. pulchra*. This conclusion is further supported by the inability of this population to crossbreed with the *B. pugnax* population.

CHAPTER ONE

INTRODUCTION

The genus *Betta* is one of the best known freshwater fish around the world mainly due to the popularity of one of its members, the Siamese fighting fish, *Betta splendens* or more commonly known as betta. This species is very popular among the aquarium hobbyist due to its beautiful appearance. It can come in a variety of colours, such as red, blue, green, purple and albino (Burkhart *et al*., 2002). It also has long flowing fins with various types of tail shape including pointed, split, round, comb and fantail which adds further splendour to its appearance (Linke, 1991).

The male Siamese fighting fish is famous for its aggressive behaviour towards other male of its own kind (Lim & Ng, 1990). Two males can fight to death when they encounter each other (Burkhart *et al*., 2002). In Thailand, the short-finned form of Siamese fighting fish is known as Pla-Kat which means biting and tearing fish. They are used in fish fighting contests as a game and betting medium (Linke, 1991).

However, all these colourful varieties of Siamese fighting fishes do not occur naturally in the wild. They are cultured varieties produced through generations of selective breeding of the *B. splendens* (Lim & Ng, 1990; Linke, 1991; Burkhart *et al*., 2002). In fact, the original wild *B. splendens* was less colourful, had shorter fins and smaller body size.

Although *B. splendens* is famous worldwide, there are actually sixty-six species of wild bettas that have already been described (Tan & Ng, 2005a, 2005b,

2006; Schindler & Schmidt, 2006). They are distributed throughout the freshwater environment of South East Asia. However, unlike the Siamese fighting fish, most of them are less colourful and less aggressive.

There are very few scientific reports on these wild bettas compared to the Siamese fighting fish which has been well documented (Forselius, 1957; Rainboth, 1996; Snekser *et al*., 2006; Karino & Someya, 2007). Although there are some information on the taxonomic status of wild bettas, the confusion about the classification of *Betta* species still exist. Some taxonomic descriptions based mainly on minor differences still remain controversial among the researchers (Schindler & Schmidt, 2006).

There are a few *Betta* species of taxonomic ambiguity which have been described without designating a holotype (Tan & Ng, 1996; Tan & Tan, 1996). One of this problematic species is the *B. pugnax*. It is reported to be polymorphic and its taxonomy is complicated (Witte & Schmidt, 1992). Although *B. pugnax* was first described from Penang Island, it can be found throughout the Peninsular Malaysia (Mohsin & Ambak, 1992; Tan & Tan, 1996). In addition, it has been recorded from Singapore, Thailand, Riau Archipelago, Sumatra and Borneo (Lim & Ng, 1990; Kottelat *et al*., 1993; Tan & Tan, 1994).

However, recent research shows that the distribution range of *B. pugnax* is much narrower (Tan & Ng, 2005a; Schindler & Schmidt, 2006). A few specimens which had formerly been identified as *B. pugnax* have been defined as a different distinct species. As such all these species which are highly similar are categorized in

one species group called the *B. pugnax* group. Eleven species of *Betta* have been described under this *B. pugnax* group (Schindler & Schmidt, 2006). These interesting findings indicate that the taxonomic status of *B. pugnax* still needs to be defined.

In this study, five populations of the *B. pugnax* group from Johor were examined. Several areas in Johor were selected as the study sites as these wetland areas with no high mountain ranges (Mohsin & Ambak, 1992) have a wide distribution of *B. pugnax* (Tan & Tan, 1996). Moreover, *B. pulchra* which was formerly identified as *B. pugnax* was described only in Johor (Tan & Tan, 1996). This species is very similar to *B. pugnax* and its taxonomic status was investigated in this study.

The three main objectives of this study were:

1) to determine the morphological quantitative variation between different members of the *B. pugnax* group from Johor.

2) to compare the reproductive behaviour of different members of the *B. pugnax* group from Johor.

3) to determine the taxonomic relationship of two populations of slightly different morphology obtained in the Mersing Forest Reserve.

CHAPTER TWO

LITERATURE REVIEW

2.1 Genus *Betta*

The genus *Betta* was established by Bleeker in 1850 (Tan & Ng, 2005a). It is classified under the family Osphronemidae. This genera is characterized by having a low count of the dorsal fin rays and unserrated lachrymal and preopercle (Witte & Schmidt, 1992). Their dorsal fin origin is over the origin of the anal fin. They have a much reduced lateral line or even lack of it in some specimens (Inger & Chin, 1962). Their caudal fin is rounded or pointed and their pelvic fin consists of a single spine and five branched rays (Rainboth, 1996).

Members of the *Betta* genus have a supplementary respiratory organ called labyrinth. This special organ is located just above the gill arches (Lim & Ng, 1990). It enables them to breathe atmospheric air which they obtain from the water surface. They can thrive in low oxygen habitat because of this respiratory structure. But they would suffocate if they were prevented to take in the atmospheric air (Linke, 1991).

Betta is the largest genera among the anabantoids (Witte & Schmidt, 1992; Rüber *et al*., 2004). Sixty-six *Betta* species have been described (Tan & Ng, 2005a, 2005b; 2006; Schindler & Schmidt, 2006). The existence of a large number of *Betta* species may due to several factors. The tropical climate of Southeast Asia provides a stable environment with various types of niches and habitats (Whitmore, 1986). The presence of various freshwater environments such as rivers, ox-bow lakes, forest streams, open country streams, hill streams, freshwater and peat swamps can support a great diversity of this genus (Kottelat & Ng, 1994). Most of the species are adapted

to survive in extreme environmental conditions such as in peat swamp with high acidity. *Betta* species also tend to be restricted to one or a few drainage systems which are delimited by various natural obstacles like mountain ranges and large rivers (Kottelat & Ng, 1994).

The systematic status of the *Betta* species is complicated (Rainboth, 1996) and still unclear due to the great diversity of this genus. Some of the species were described based mainly on minor differences which have not been universally accepted among researchers in this field (Schindler & Schmidt, 2006). It is believed that the *Betta* genus is still in a state of rapid evolution, being a young group of species (Forselius, 1957).

2.2 *Betta* Species Group

Several *Betta* species groups are generally recognized. They were classified based mainly on superficial similarities, behavioural and morphological characters. However, each group may or may not be a monophyletic lineage (Tan & Ng, 2005a). The *Betta* genus was first divided into two groups by Schaller (1985) and Schaller & Kottelat (1989). These two groups were the mouthbrooders and bubblenest brooders. Schaller (1985) further separated the bubblenest brooders into *B. splendens* and *B. bellica* groups based on superficial similarities, behavioural and physiological adaptations to different environments. On the other hand, Vierke (1991) suggested that the *Betta* genus could be separated into three species groups based almost entirely on ethological characters and overall body shape. These three species groups were *B. splendens*, *B. pugnax* and *B. macrostoma* groups.

However, Witte & Schmidt (1992) established fifteen species groups based on easily recognizable behavioural and morphological character, and to a large extent on details of the head and body patterns which were usually diagnostic. These were *B. unimaculata, B. patoti, B. edithae, B. picta, B. akarensis, B. anabatoides, B. pugnax, B.* species E*, B. splendens, B. coccina, B. foerschi, B. macrostoma, B. dimidiata, B. bellica* and *B. rubra* groups. Ng & Kottelat (1994) increased the number of the species groups by separating the *B. waseri* group which was positioned under the *B. anabatoides* group in the earlier grouping of Witte & Schmidt (1992) on the basis of morphological, meristic and colour characters.

Tan & Ng (2005a) later modified the above species grouping by combining *B. patoti, B. unimaculata* and *B. macrostoma* groups into the *B. unimaculata* group. They also redefined the *Betta* species E group as a member of the *B. pugnax* group. Furthermore, the *B. rubra* group was placed under the *B. foerschi* group. Therefore, Tan & Ng (2005a) recognized only thirteen species groups.

Schindler & Schmidt (2006) further reduced the number of the species groups by grouping *B. edithae* into the *B. picta* group. They recognized only twelve species groups and sixty-six nominal species (Table 2.1). In this study, the number of species groups and the included species follow Schindler & Schmidt (2006).

2.3 *Betta pugnax* Group

The members of the *Betta pugnax* group are characterized by the following set of characters: a relatively large head, 28-40% in standard length; usually brown with greenish to bluish iridescent spots on body scales when alive; male with

Table 2.1 *Betta* species list (Schindler & Schmidt, 2006)

***Betta bellica* group**
Betta bellica Sauvage, 1884
Betta simorum Tan & Ng, 1996

***Betta pugnax* group**
Betta pugnax (Cantor, 1850)
Betta fusca Regan, 1910
Betta schalleri Kottelat & Ng, 1994
Betta pulchra Tan & Tan, 1996
Betta breviobesus Tan & Kottelat, 1998
Betta lehi Tan & Ng, 2005
Betta stigmosa Tan & Ng, 2005
Betta cracens Tan & Ng, 2005
Betta raja Tan & Ng, 2005
Betta ferox Schindler & Schmidt, 2006
Betta apollon Schindler & Schmidt, 2006

***Betta akarensis* group**
Betta akarensis Regan, 1910
Betta balunga Herre, 1940
Betta chini Ng, 1993
Betta pinguis Tan & Kottelat, 1998
Betta aurigans Tan & Lim, 2004
Betta ibanorum Tan & Ng, 2004
Betta obscura Tan & Ng, 2005
Betta antoni Tan & Ng, 2006

***Betta unimaculata* group**
Betta unimaculata (Popta, 1905)
Betta macrostoma Regan, 1910
Betta patoti Weber & de Beaufort, 1922
Betta ocellata de Beaufort, 1933
Betta gladiator Tan & Ng, 2005
Betta pallifina Tan & Ng, 2005
Betta ideii Tan & Ng, 2006
Betta compuncta Tan & Ng, 2006

***Betta albimarginata* group**
Betta albimarginata Kottelat & Ng, 1994
Betta channoides Kottelat & Ng, 1994

***Betta dimidiata* group**
Betta dimidiata Roberts, 1989
Betta krataios Tan & Ng, 2006

***Betta picta* group**
Betta picta (Valenciennes, in Cuvier & Valenciennes, 1846)
Betta taeniata Regan, 1910
Betta edithae Vierke, 1984
Betta simplex Kottelat, 1994
Betta falx Tan & Kottelat, 1994
Betta prima Kottelat, 1994
Betta enisae Kottelat, 1995
Betta pallida Schindler & Schmidt, 2004

***Betta splendens* group**
Betta splendens Regan, 1910
Betta smaragdina Ladiges, 1972
Betta imbellis Ladiges, 1975
Betta stiktos Tan & Ng, 2005

***Betta coccina* group**
Betta coccina Vierke, 1979
Betta tussyae Schaller, 1985
Betta persephone Schaller, 1986
Betta rutilans Witte & Kottelat, in Kottelat, 1991
Betta brownorum Witte & Schmidt, 1992
Betta livida Ng & Kottelat, 1992
Betta miniopinna Tan & Tan, 1994
Betta burdigala Kottelat & Ng, 1994
Betta uberis Tan & Ng, 2006

***Betta waseri* group**
Betta waseri Krummenacher, 1986
Betta hipposideros Ng & Kottelat, 1994
Betta spilotogena Ng & Kottelat, 1994
Betta tomi Ng & Kottelat, 1994
Betta chloropharynx Kottelat & Ng, 1994
Betta renata Tan, 1998
Betta pi Tan, 1998

***Betta foerschi* group**
Betta foerschi Vierke, 1979
Betta strohi Schaller & Kottelat, 1989
Betta rubra Perugia, 1893
Betta mandor Tan & Ng, 2006

***Betta anabatoides* group**
Betta anabatoides Bleeker, 1851

iridescent blue, green or greenish blue opercle scales; juvenile and female usually with a light brown background displaying a central and second central stripe, with a caudal peduncle spot; head rhombic when viewed from above, with or without chin bar; fins of mature specimens pointed, often elongated, caudal fin lanceolate, male may exhibit caudal fin transverse bars. All members are paternal mouthbrooders (Tan & Ng, 2005a).

Tan & Ng (2005a, 2005b) recognized eleven species from the *B. pugnax* group. They are *B. pugnax*, *B. fusca*, *B. schalleri*, *B. prima*, *B. enisae*, *B. pulchra, B. breviobesus, B. stigmosa, B. lehi, B. raja* and *B. cracens*. However, Schindler & Schmidt (2006) placed the *B. prima* and *B. enisae* into the *B. picta* group because these two species share a low number of lateral scales, a comparatively low count of anal fin rays and a relatively small maximum size with *B. picta*. At the same time, Schindler & Schmidt (2006) included two new species in the *B. pugnax* group, *B. ferox* and *B. apollon*. Hence, the *B. pugnax* group as according to these authors also consists of eleven species but with two different members from the grouping proposed by Tan & Ng (2005a, 2005b). They are *B. pugnax, B. fusca, B. schalleri, B. pulchra, B. breviobesus, B. stigmosa, B. lehi, B. raja, B. cracens, B. ferox* and *B. apollon*.

2.4 Species of *Betta pugnax* Group from Johore

2.4.1 Distribution

The members of *Betta pugnax* group are widely distributed in South East Asia. However, referring to Tan & Tan (1996), there are only two described species from *B. pugnax* group in Johore namely *B. pugnax* and *B. pulchra*. Tan & Tan (1996)

sampled specimens of *B. pugnax* from various areas in Peninsular Malayisa including Johor. Their specimens were collected from: 1) Sungai Anak Jasin, Ulu Endau; 2) Sungai Tementang, Kota Tinggi; 3) Sungai Mupor along Kota Tinggi-Mersing road; 4) 100 m north of road marker 175 km on Johor Bahru-Kuantan road (North of Mersing); 5) Swamp near Mersing; 6) 14th mile on Muar-Yong Peng Road; 7) Mawai district; 8) Gunung Pulai Reservoir; 9) stream via access road to Pekan Nenas from Gunung Pulai; 10) stream draining from Gunung Pulai Reservoir, Pontian; 11) Gunung Panti foothills; 12) Air Hitam, Parit Botak; 13) Kampung Parit Tekong, Sri Bunian, Pontian, 14) 4 km towards Pontian Kechil from Sri Bunian and 15) Kampung Jasa Sepakat, Pontian (Tan & Tan, 1996). They concluded that the specimens which were collected from Kampung Parit Tekong, Sri Bunian; 4 km towards Pontian Kechil from Sri Bunian; Kampung Jasa Sepakat in Pontian were a distinct species and described them as *B. pulchra*. The other specimens were all identified as *B. pugnax* (Tan & Tan, 1996).

2.4.2 Morphology

2.4.2 (a) *Betta pugnax*

The main characters of *Betta pugnax* are its relatively large head, 2.5-3.6 times in standard length; iris without broad iridescent patches; body usually brown with greenish to bluish iridescent spots on the scales when alive, male usually with the iridescent operculum scales; juveniles and females usually with a light brown background displaying two dark bands running transversely across the body converging at the base of the caudal peduncle with a dark spot; body stout, body depth 2.7-3.6 times in standard length; juveniles generally more slender with proportionately larger eyes; head rhombic when viewed from above; caudal fin of

mature fish pointed, often elongated, caudal broadly lanceolate (Figure 2.1.a); chin bar usually present but sometimes not visible (Tan & Tan, 1996).

2.4.2 (b) *Betta pulchra*

Betta pulchra is a stouter fish than *B. pugnax*. Its body depth is 25.9-36.9% in standard length. Live *B. pulchra* have more iridescent scales on the body than that of the *B. pugnax*. The caudal fin of *B. pulchra* is narrowly lanceolate (Figure 2.1.b) compared to the broadly lanceolate caudal fin of *B. pugnax.* The dorsal, caudal, anal and pelvic fins of *B. pulchra* are proportionately longer than those of *B. pugnax*. *Betta pulchra* is only known from the blackwaters in Pontian, Johor (Tan & Tan, 1996).

2.4.3 Habitat

Betta pugnax usually live in streams on hills and around the foothills. These are clear water streams ranging from sluggish to fast flowing. The habitat typically had very little or no submerged vegetation in the habitat which is usually with sandy bottoms covered with rocks. *B. pugnax* are always found in and at the peripheries of stagnant areas with pH range averaging 7.4 (7.1-7.6) and water depth ranging from 10 to 80 cm. There were no other belontiids sympatric with *B. pugnax* (Tan & Tan, 1996).

Betta pulchra live in blackwater habitats with pH ranging from 3.9-4.2. This species is common found only in the overgrown bank vegetation of irrigation canals. The other sympatric belontiids were *B. bellica, B. imbellis, Belontia hasseltii,*

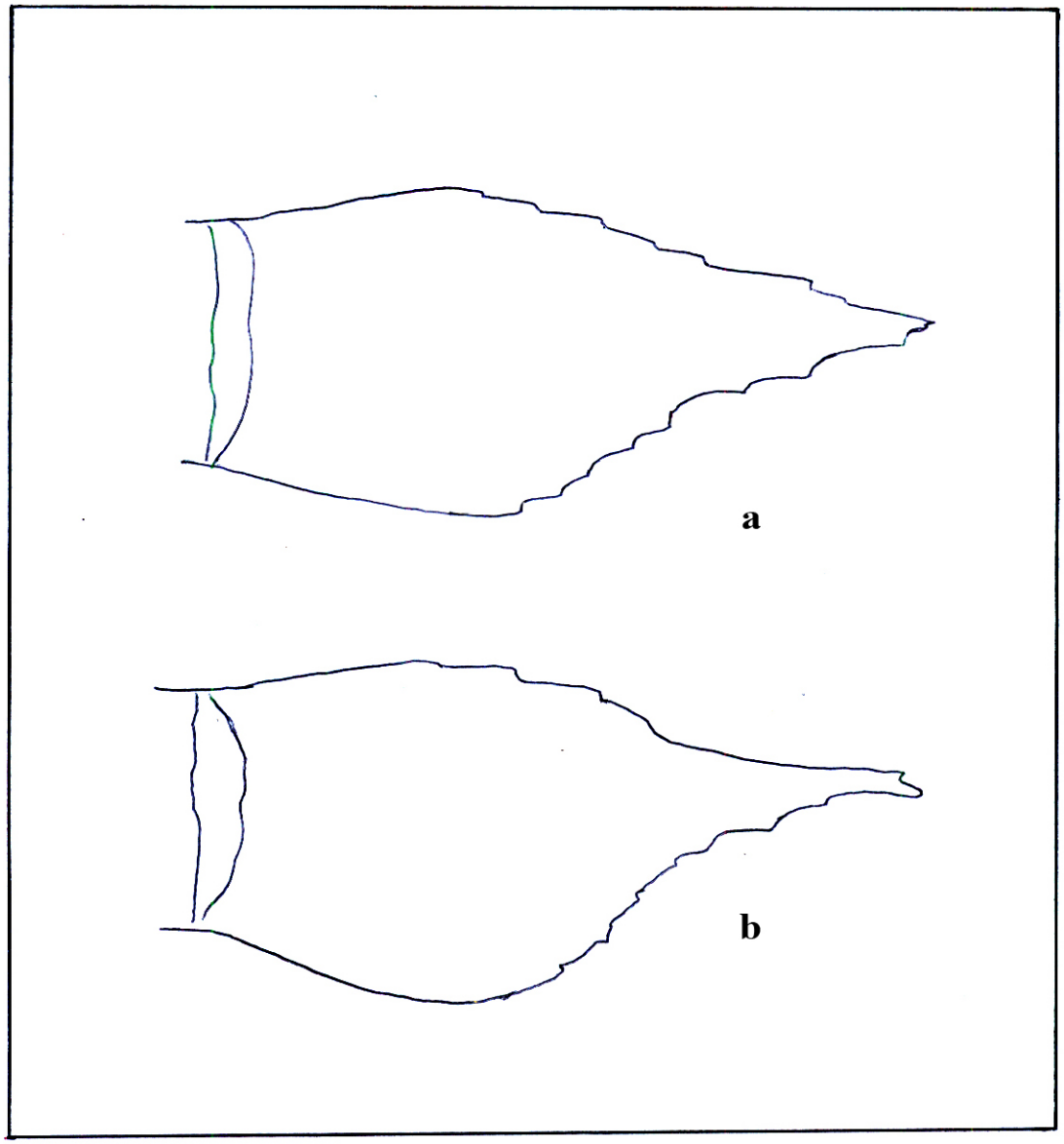

Figure 2.1 Schematic diagram of caudal fins adopted from Tan & Tan (1996).
a) broadly lanceolate caudal fin (*B. pugnax*).
b) narrowly lanceolate caudal fin (*B. pulchra*).

Sphaerichthys osphromenoides, Parosphromenus sp. and *Trichopsis vittata* (Tan & Tan, 1996).

2.5 Identification of *Betta* Species

Traditionally, the identification of the *Betta* species has been mainly based on traditional morphological technique namely meristic and traditional morphometric techniques (Witte & Schmidt, 1992). The colouration is also useful in distinguishing the live specimens (Tan & Ng, 2005a). In this study, truss network morphometric techniques were also utilized to investigate the degree of variation among populations and species.

2.5.1 Quantitative Morphological Techniques

Quantitative morphological techniques could basically be classified into meristic and morphometric. These two numerical techniques are important in the process of scientific description of fishes (Taniguchi *et al*., 1996; Turan, 1999; Loy *et al*., 2000; Akhter *et al*., 2003; Barriga-Sosa *et al*., 2004; Pinheiro *et al*., 2005).

2.5.1 (a) Meristic Technique

The meristic approach has been widely used to differentiate among species (Barriga-Sosa *et al*., 2004), populations or group within species (Cabral *et al*., 2003; Pinheiro *et al*., 2005). It involves countable anatomical structures like the fin ray counts, number of spines and scales counts. However, meristic characters are generally conservative for the *Betta* identification, especially within the *Betta* species group. Species distinction based only on the presence, absence or number of spines in the dorsal and anal fins are often unreliable (Tan & Tan, 1996). However,

meristic technique was conducted in this study for comparisons with previous studies.

2.5.1 (b) Traditional Morphometric Technique

Traditional morphometric involves measuring the distance between physical features like head length, body depth and caudal fin length. It is a useful tool for testing and graphically displaying difference in shape when combined with multivariate statistical procedures (Loy *et al*., 1993; Rohlf & Marcus, 1993; Rohlf *et al*., 1996). Traditional morphometric have been useful in distinguishing among certain *Betta* species, but characters of certain species groups are often difficult to define objectively and may possess some geographic variation (Tan & Ng, 2005a). However, traditional morphometric technique was conducted in this study for comparisons with previous works.

2.5.1 (c) Truss Network Morphometric Technique

The truss network morphometric technique is an extension of the traditional morphometric. It is a powerful technique for investigating morphological differentiation and was introduced by Strauss & Bookstein (1982). It involves measuring the distance between many homologous landmarks which are chosen over the entire length on the surface of the fish. The shape of the fish is reconstructed when the homologous landmarks are connected. This truss network technique is deemed more accurate to the traditional technique because it is thus more sensitive to differences between and within fish species not detectable by the traditional morphological technique (Strauss & Bookstein, 1982; Cavalcanti *et al*., 1999; Siti Azizah *et al*., 2001; Jayasankar *et al*., 2004; Rutaisire *et al*., 2005). It is

also able to detect the differences in the shape of individuals which are known to be influenced by genetics and environment. Unlike traditional morphometrics where character sets tend to be aligned to the same horizontal axes, the truss network morphometric technique poses no restriction on the directions of variation and localization of the shape change. It is much more effective in capturing information about the shape of the fish (Schweigert, 1990; Cavalcanti *et al*., 1999; Turan, 1999; Cadrin, 2000; Palma & Andrade, 2002; Kassam *et al*., 2002; Jayasankar *et al*., 2004).

2.5.2 Colouration

Colouration of the *Betta* species could be useful in distinguishing live specimens. However, colour may vary depending on maturity, sex, reproductive condition and geographic variation. Large and old specimens tend to be darker, which may mask body and opercle patterns (Kottelat *et al*., 1993; Kottelat & Ng, 1994; Roberts, 1989). The identification through colouration may be difficult to use if the investigator is unfamiliar with the infraspecific variation (Kottelat *et al*., 1993; Kottelat & Ng, 1994; Roberts, 1989). However, initial identification of field specimens are done on colouration before further analyses were carried out in the laboratory.

2.6 Statistical Analysis

There are various types of statistical methods to analyse the differences and relationships among populations. The statistical methods widely applied in meristic and morphometric analyses are univariate analysis of variance (ANOVA) and multivariate analyses of principal component analysis (PCA) and discriminant function analysis (DFA).

2.6.1 Analysis of Variance (ANOVA)

Analysis of variance or ANOVA is one of the most powerful statistical techniques used to test for significant differences between two or more sample means. There are several types of ANOVA such as one-way ANOVA, two-way ANOVA and so on. The one-way ANOVA is used when the study involves a single independent variable. The two-way ANOVA is used when there are two independent variables. A post hoc test is always carried out to interpret the results when significant results with *p*-value of <0.05 are obtained from the ANOVA to determine population groups which are significantly different from each other (Dytham, 2003).

2.6.2 Principal Component Analysis (PCA)

Principal component analysis (PCA) is a concise statistical method to investigate the maximum differences between individuals without assigning into *a priori* grouping. It generates a set of principal components by weighting all the available variables. The first component explains the most variation, the second explains the next most variation, and so on. Investigation of the first few components will show which variables contribute most to the differences between individuals (Dytham, 2003).

In morphometric analysis, it is usually the case that individual specimens will vary in size. The first principal component will always account for size and it is often employed as a method for removing size from the analysis. The second and the rest of the principal components are usually interpreted as shape component (Dytham, 2003). Unlike the first component, which is positively correlated to all

dimensions, the second principal as well as other components are positively correlated to some variables, and negatively correlated with others (Cadrin, 2000).

2.6.3 Discriminant Function Analysis (DFA)

Discriminant function analysis (DFA) is a multivariate extension of analysis of variance (ANOVA) to study variation and covariation among taxonomic groups (Fisher, 1936). DFA works in very much the same way as PCA. However, DFA requires that the individuals be assigned into *a priori* grouping. DFA tests significance of group differences by deriving a weighted combination of variables which maximize the differences between groups rather than individuals as in the case with PCA. The weightings could then be used on individuals that are not pre-assigned to a group to provide a probability of them belonging to each of the possible groups (Dytham, 2003).

2.7 Reproductive Behaviour

Betta pugnax and its allied species are mouthbrooders (Tan & Ng, 2005a). Linke (1991) described the reproductive behaviour of captive *B. pugnax*. His observation showed that the *B. pugnax* always spawn near the tank bottom. The male would embrace the female and the eggs merge to fall on to the slightly arched anal fin of the male where the eggs would be picked up by the female and held inside its mouth. The spawning phases took place at quite lengthy intervals, almost always at the same place in the aquarium. Between the individual bouts of the courtship, the female would spit out the eggs in front of the male one at a time for the male to snap up and retain. On occasions the male may spit back the eggs at the female so that something of a "ball game" would develop. The spawning process

could take up to 5 hours after which the male would retire into the protection of a densely planted zone after the spawn. About 10 days later, the fry would be fully developed and released by the male (Linke, 1991).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Specimen Collection

The specimens were collected based on the description of *Betta pugnax* group by Tan & Ng (2005a) from four different locations in Johor by using scoop net or hook and line. These four locations were: 1) Mersing Forest Reserve; 2) Kampung Sinar Harapan, Pekan Nenas; 3) Kampung Parit Jepon, Muar and 4) Parit Lapis 2, Sri Bunian (Table 3.1 and Figure 3.1). Sampling was conducted from August 2005 to December 2006.

Two morphologically different populations were caught in the Mersing Forest Reserve. One population comprising of individuals with absence of second postorbital stripe was labeled as I and the other population with presence of second postorbital stripe was labeled as II. The population from Kampung Sinar Harapan, Pekan Nenas was labeled as III. The populations from Kampung Parit Jepon, Muar and Parit Lapis 2, Sri Bunian were labeled as IV and V respectively.

The pH, temperature and conductivity of each location were measured. Sympatric species of fish occurring in each population were also recorded. River/stream characteristic such as colour, movement, and depth of water as well as types of bottom were also recorded. Photos of each location were taken.

Twenty individuals of each population were preserved in 10% formalin for the meristic, traditional and truss network morphometric measurements. Five pairs of each population were kept in aquariums for the reproductive behaviour

Table 3.1 Locality, coordinates and number of individuals analysed of populations I, II, III, IV and V.

No	Populations	Number of individuals analysed	Locality	Coordinates
1.	I	20	Mersing Forest Reserve	02º25'N 103º77'E
2.	II	20	Mersing Forest Reserve	02º25'N 103º77'E
3.	III	20	Kampung Sinar Harapan	01º30'N 103º30'E
4.	IV	20	Kampung Parit Jepon	02º00'N 102º45'E
5.	V	20	Parit Lapis 2	01º27'N 103º26'E

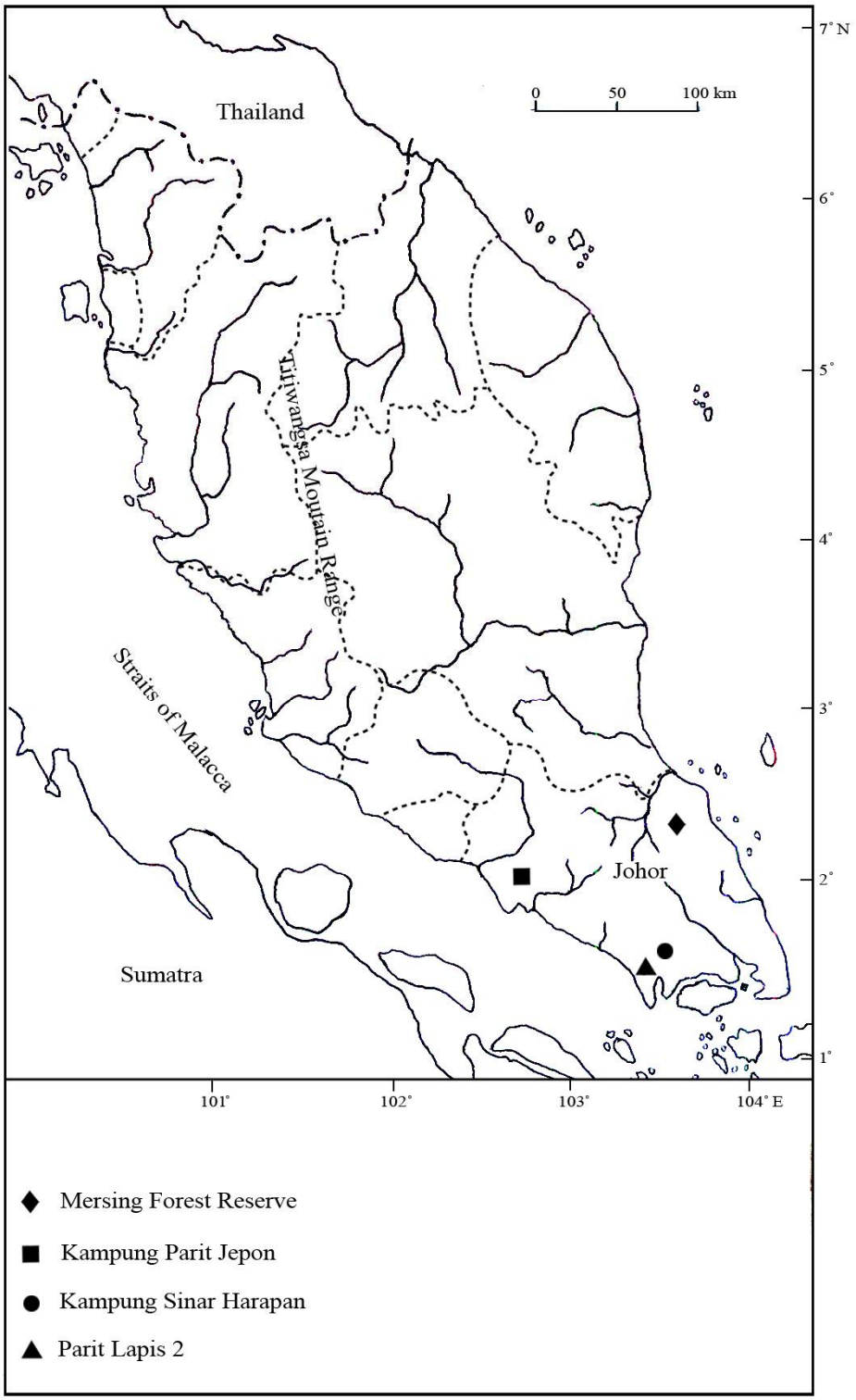

Figure 3.1 Location of sampling sites in Johor.

observation. Specimens of each population were identified based on the taxonomic key as described by Tan & Tan (1996); Tan & Ng (2005a, 2005b, 2006); Schindler & Schmidt (2006).

3.2 Meristic and Traditional Morphological Techniques

The meristic and traditional morphometric measurements followed Witte & Schmidt (1992) except that all the measurements were taken across a straight-line between two landmarks, the predorsal scale counts were counted continuously and the snout length was measured from the tip of the snout to the anterior margin of orbit as according to Ng & Kottelat (1994).

3.2.1 Meristic

All counts were done on the left side of the fish unless the specimens were severely damaged. A needle with a pointed end and a pair of forceps was used in handling and counting the specimens. Strong transmitted light was used when counting. Ten variables as listed in Table 3.2 and Figure 3.2 were counted.

As the squamation pattern of the *Betta* is very conservative, the reference points for counts are designated as indicated in Figure 3.2. The lateral row is designated row '0'. The rows above and below are numbered +1, +2, +3, +4 and +5 or -1, -2, etc., respectively. Thus, row +1 corresponds to the upper section and row-1 to the lower section of the vestigial, interrupted lateral line.

Table 3.2 Description of meristic variables.

No.	Variables	Code of variables	Description of variables
1.	Dorsal fin rays	D	All rays are counted. The unarticulated fin rays including the spine are given in roman numerals. The articulated fin rays are given in arabic numerals.
2.	Anal fin rays	A	All rays are counted. The unarticulated fin rays including the spine are given in roman numerals. The articulated fin rays are given in arabic numerals.
3.	Pectoral fin rays	P1	All rays are counted including the single dorsalmost spine or spinous ray and the single ventralmost unarticulated ray which are very variable in length. The fin ray counts are given in arabic numerals.
4.	Pelvic fin rays	P2	The fin ray counts are always invariable. They consist of a single spine, a generally filamentous ray and four branched rays. The spine count is given in roman numerals. The filamentous ray and branched rays are given in arabic numerals.
5.	Caudal fin rays	C	The upper unarticulated (rudimentary) rays, articulated (principal) rays attached to the upper + lower part of the hypural complex, and the lower unarticulated rays are counted. The unarticulated rays are given in roman numerals and the articulated rays are given in arabic numerals.
6.	Lateral scales	ls	Scales behind the pored scales situated anterior-dorsally of the opercular slit to the caudal flexure.
7.	Predorsal scales	PRS	Scales at the row +5 from the origin of the dorsal fin to the head.
8.	Subdorsal scales	SDS	Scales at row +3 lying below the dorsal fin.
9.	Postdorsal scales	POS	Scales at the row +4 after the dorsal fin. These are counted from a pair of scale behind the last dorsal ray to the caudal flexure.
10.	Transversal scales	ts	Scales along the column directly anterior of the dorsal (forward) and the below row, backward to the anal fin base (excluding those scales with horizontal margin with anal sheath).

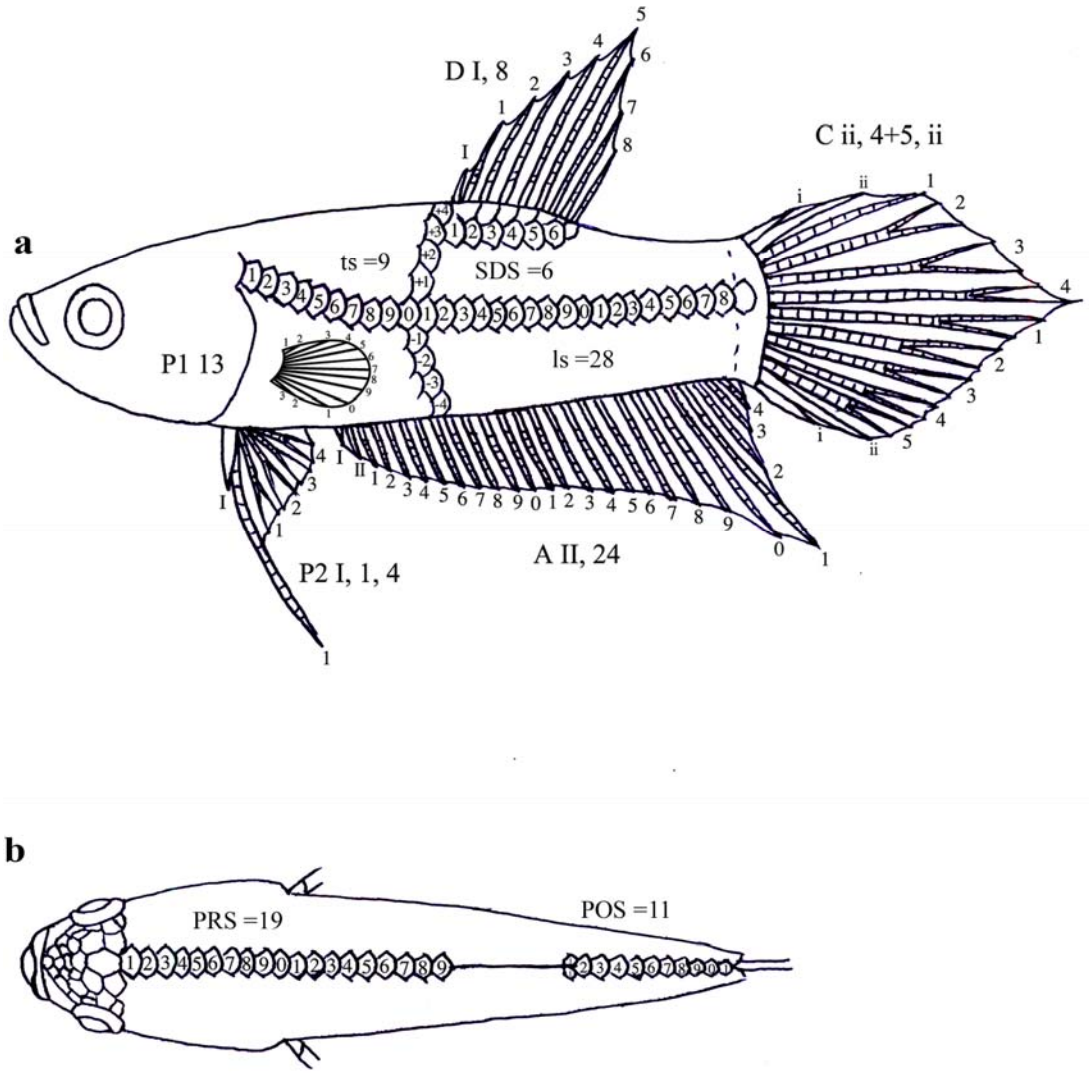

Figure 3.2 Diagram of meristic variables. a) lateral view; b) dorsal view
D= dorsal fin rays; A= anal fin rays; P1= pectoral fin rays; P2= pelvic fin rays; C= caudal fin rays; ls= lateral scales; PRS= predorsal scales; SDS= subdorsal scales; PDS= postdorsal scales; ts= transversal scales

3.2.2 Traditional Morphometric

The distances were measured on the left side of a specimen with a digital caliper reading to the nearest 0.01 mm. Measurements were performed on a styrofoam board to ease the measuring procedure. Twenty variables were measured. Descriptions of the variables are as in Table 3.3. The measurements were taken as a straight-line between two landmarks. The corresponding landmarks are noted in Figure 3.3.

3.3 Truss Network Morphometric Technique

Measurements were conducted based on truss network anchored at 10 homologous landmarks labeled 1-10 (Figure 3.4). This resulted in 22 linear measurements including standard length. Four quadrilateral cells, each having two internal diagonals as seen in the diagram were produced. These measurements were performed to the nearest 0.01 mm using a digital caliper. Descriptions of the variables are as in Table 3.4.

3.4 Data Analysis

The data obtained from the meristic, traditional morphometric and truss network morphometric techniques were analysed by a one-way analysis of variance (one-way ANOVA) and multivariate analyses of principal component analysis (PCA) and discriminant function analysis (DFA) using the Statistical Package for Social Sciences (SPSS) version 11.5 software for windows.