คาริโอไทป์และการกระจายของบริเวณนิวคลิโอลัสออกาไนเซอร์ของปลาตะเพียน 4 ชนิดในประเทศไทย

นางสาวจันทิมา ปิยะพงษ์

KARYOTYPES AND DISTRIBUTION OF NUCLEOLUS ORGANIZER REGIONS

IN FOUR CYPRINID SPECIES FROM THAILAND

Miss Chantima Piyapong

Thesis Title   Karyotypes and distribution of nucleolus organizer regions in four
                      cyprinid species from Thailand
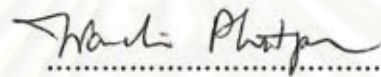
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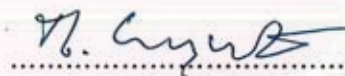
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จันทิมา ปิยะพงษ์: คาริโอไทป์และการกระจายของบริเวณนิวคลิโอลัสออกาไนเซอร์ของปลา ตะเพียน 4 ชนิดในประเทศไทย (KARYOTYPES AND DISTRIBUTION OF NUCLEOLUS ORGANIZER REGIONS IN FOUR CYPRINID SPECIES FROM THAILAND) อ. ที่ปรึกษา: ศ. ดร. ทศพร วงศ์รัตน์, อ.ที่ปรึกษาร่วม: รศ. ดร. วิเชียร มากตุ่น, 85 หน้า. ISBN 974-334-866-2.

Rainboth(1996b) ได้จัดจำแนกปลาในสกุล *Puntius* Hamilton,1822 แต่เดิมโดย Smith(1945) ออกไว้ ใน 4 สกุลคือ *Puntius* , *Systomus* , *Barbodes* และ *Hypsibarbus* โดยอาศัยความแตกต่างที่เห็นได้ชัดทาง ลักษณะภายนอก แต่เนื่องจากคาริโอไทป์และการกระจายของบริเวณนิวคลิโอลัสออกาไนเซอร์ (NORs) ซึ่งเป็นการ ศึกษาทางด้านเซลล์อนุกรมวิธานนั้นน่าจะเป็นประโยชน์ในการช่วยยืนยันการจัดจำแนกปลาดังกล่าว ดังนั้นจึงได้ ทำการศึกษาเปรียบเทียบสัณฐานวิทยาภายนอกที่สำคัญบางประการและเน้นไปที่การศึกษาเปรียบเทียบคาริโอ ไทป์และการกระจายของ NORs ของปลาตะเพียน 4 ชนิดต่างสกุลกันดังกล่าวได้แก่ ปลาตะเพียนทราย(*Puntius brevis*), ปลาแก้มช้ำ(*Systomus orphoides*), ปลาตะเพียนขาว(*Barbodes gonionotus*) และปลาตะพาก (*Hypsibarbus wetmorei*)

ปลาที่ศึกษาในครั้งนี้นำมาจากแหล่งน้ำธรรมชาติ ของระบบลุ่มน้ำเจ้าพระยาทางภาคกลางของประเทศ- ไทยซึ่งแต่ละชนิดนำมาจากจุดเดียวกันของแหล่งน้ำเป็นจำนวนปลาชนิดละ 16-20 ตัว 10 ตัวแรกของแต่ละชนิดได้ นำมาศึกษาด้านสัณฐานวิทยาโดยการวัดและการนับลักษณะภายนอกบางประการ ส่วนปลาตะเพียนขาวและปลา ตะพากที่เหลืออีก 10 ตัว,ปลาตะเพียนทราย 8 ตัวและปลาแก้มช้ำ 6 ตัวนั้นนำมาศึกษาคาริโอไทป์และการกระจาย ของ NORs

จากข้อมูลที่รวบรวมได้ในการศึกษาครั้งนี้พบว่าสัณฐานวิทยาบางประการของชนิดตัวแทนปลาทั้ง 4 สกุล นั้นมีความแตกต่างกันชัดเจนในระดับสกุล ดังที่ยอมรับกันแล้วและน่าจะยืนยันได้อีกจากผลการศึกษาคาริโอไทป์ และการกระจายของ NORs ซึ่งพบว่าแม้ปลาทั้ง 4 ชนิดจะมีจำนวนโครโมโซมแบบดิพลอยด์เท่ากันคือ 50 แต่ก็ มีชนิดของโครโมโซม, แขนโครโมโซม, จำนวนและตำแหน่งของ NORs ที่หลากหลายมากเช่นกันดังนี้คือ ปลา ตะเพียนทรายมีคาริโอไทป์ประกอบด้วยโครโมโซมแบบ 2m, 2sm, 2st และ 44a โดยมีจำนวนแขนของโครโมโซม (NF) เท่ากับ 54 และ NORs มี 1 คู่ที่ส่วนปลายสุดของโครโมโซมแขนสั้นชนิด st , ปลาแก้มช้ำมีคาริโอไทป์ประกอบ ด้วยโครโมโซมแบบ 12m, 20sm, 4st และ 14a โดยมี NF เท่ากับ 82 และ NORs มี 1 คู่ที่ส่วนปลายสุดของ โครโมโซมแขนสั้นชนิด sm ,ปลาตะเพียนขาวมีคาริโอไทป์ประกอบด้วยโครโมโซมแบบ 6m, 16sm, 6t และ 22a โดยมี NF เท่ากับ 72 และ NORs มี 2 คู่ที่ส่วนปลายสุดของโครโมโซมแขนสั้นชนิด smและที่ส่วนปลายสุดของ โครโมโซมแขนสั้นชนิด a และส่วนปลาตะพากมีคาริโอไทป์ประกอบด้วยโครโมโซมแบบ 12m, 12sm, 4st และ 22a โดยมี NF เท่ากับ 74 และ NORs มี 1 คู่ที่ส่วนปลายสุดของโครโมโซมแขนยาวชนิด a

ภาควิชา	ชีววิทยา	ลายมือชื่อนิสิต	
สาขาวิชา	สัตววิทยา	ลายมือชื่ออาจารย์ที่ปรึกษา	
ปีการศึกษา	2542	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม	

   CHANTIMA PIYAPONG: KARYOTYPES AND DISTRIBUTION OF NUCLEOLUS ORGANIZER REGIONS IN FOUR CYPRINID SPECIES FROM THAILAND. THESIS ADVISOR: PROF. THOSAPORN WONGRATANA, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. WICHIAN MAGTOON, Ph.D., 85 pp. ISBN 974-334-866-2.

   Rainboth(1996b) has varified all *Puntius* species of Smith(1945) into 4 genera, *Puntius* , *Systomus*, *Barbodes* and *Hypsibarbus* by taking their distinctive external morphological differences into account. Since cytotaxonomy by the studying of karyotypes and distributions of nucleolus organizer regions (NORs) might provide possible useful data to support such taxonomic problem or vice versa. This study was therefore designed to do comparative study by examining some external morphological features but emphasizing on the comparison of karyotypes and distributions of NORs of a representative cyprinid species of those above 4 genera, they are *Puntius brevis*, *Systomus orphoides*, *Barbodes gonionotus* and *Hypsibarbus wetmorei*, respectively.

   The fish materials in this study were obtained from middle plain of the Chao Phraya river system. Each species was taken at the same place as many as enough for selection. For morphometric and meristic studies ten specimens of each species were encountered. Whereas another ten specimens of both *Barbodes gonionotus* and *Hypsibarbus wetmorei* , eight specimens of *Puntius brevis* and 6 specimens of *Systomus orphoides* were used for the studies of karyotypes and distribution of NORs.

   From the obtainable data on the external morphology of the sole representative species of the four genera, they exhibit a number of distinctive feature to render the agreeable separation of the species at the generic level. To support the above taxonomic thought, this further study on karyotypes and distributions of NORs have shown that although all the four species of different genera has similarly 50 diploid chromosome number(2n), however, the combinations of chromosome types, arm number (NF), number and location of NORs are likely different. For *Puntius brevis*, the karyotypes comprise of 2m, 2sm, 2st and 44a; 54 for NF and NORs have one pair which locate on the satellited regions of a subtelocentric pair on short arms. *Systomus orphoides*, the karyotypes comprise of 12m, 20sm, 4st and 14a; 82 for NF and NORs have one pair which locate on the satellited regions of a submetacentric pair on short arms. *Barbodes gonionotus*, the karyotypes comprise of 6m, 16sm, 6st and 22a; 72 for NF and NORs have two pairs which locate on the satellited regions of a submetacentric pair on short arms and on the satellited regions of an acrocentric pair on short arms. *Hypsibarbus wetmorei*, the karyotypes comprise of 12m, 12sm, 4st and 22a; 74 for NF and NORs have one pair which locate on the satellited regions of an acrocentric pair on long arms.

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Chapter 1

Introduction

Fish exhibit enormous diversity in their morphology, in the habitats they occupy, and in their biology (Nelson, 1994). Taxonomy, roughly defined as the study of the classification with latin name of biological diversity, will always remain an important and active branch of biology (Wongratana, 1993).

The family Cyprinidae is the largest family of freshwater fishes or various members of this family are important as food fish, as aquarium fish and in biological research (Nelson, 1994). Smith (1945) who studied the fresh-water Fishes of Siam, or Thailand reported that the Thai Cyprinidae include as much as the largest family and being the 54 genera. Especially genus *Puntius* which included many species members and being found generally all over the country. According to the Smith (1945)'s monograph, Thai fish species of the genus *Puntius* were identified or named accordingly to Weber and de Beaufort (1916) who did not care about barbel numbers. Since then, therefore, numbers of some cyprinid species in Southeast Asian water were identified as member of the genus *Puntius*. However, recently, Rainboth (1996b) who studied fishes of the Cambodian Mekong divided the *Puntius* of Weber and de Beufort(1916), or Smith (1945) to four genera according to fin ray characters, barbel numbers, gill raker numbers, lower lip characters and proportion of anal-fin base length in relation to head length. From these principle criteria the former *Puntius* spp.has been varified and variously belonged to *Puntius, Systomus, Barbodes* and *Hypsibarbus.*

Scientifically, comparative karyology has become a useful tool in fish systematic studies (Buth, Dowling and Gold, 1991 cited in Magtoon and Arai, 1993). In this benefit, Nucleolus Organizer Regions (NORs) phenotypes which one type of cytotaxonomy can be utilized as characters for testing or inferring hypotheses of cyprinid relationships (Jenkin, Li and Gold, 1992). Because NOR-bands with silver (Ag) represent chromosomal sites of nuclear genes that encode 18S and 28S ribosomal RNAs (Gold et al., 1990). This banding uses silver nitrate solution to stain chromosomes. Historically,

NOR-bearing chromosome studying in Thailand, there was only Magtoon and Arai (1993) who studied and rendered the method for fish.

Since fishes of the genus *Puntius* belong to many species and taxonomically encounter different combinations of characters, for many instances they are divided by sub-groups, however this solution many of generic classification is still generally unsatisfied. For Rainboth' s (1996b) idea proof, this studying was aimed to look for the better ideas in the former *Puntius* which has identified by Rainboth(1996b) into four genera. The purpose of this study is to use the micro-technique for representative species of the four genera, namely, golden little barb (*Puntius brevis*), red-cheek barb (*Systomus orphoides*), common silver barb (*Barbodes gonionotus*) and golden belly barb (*Hypsibarbus wetmorei*). Basically, all four species are common, could be collected easily in any river of Thailand and are commercially important fishes. This study has been designed to make comparison study of some important morphology among the four species but this are treated as a minor study, while the major study is the comparison of karyotypes and chromosomal NOR-phenotypes. It is hope that this study could give information and betters ideas to develop biological taxonomy among members of the family Cyprinidae.

Objectives

1. To make the comparison of some important morphology among four cyprinid fishes of different genera, namely, *Puntius brevis*, *Systomus orphoides*, *Barbodes gonionotus* and *Hypsibarbus wetmorei*.
2. To make the comparison of karyotypes and chromosomal NOR-phenotypes of the four cyprinid fishes.

Anticipated benefits

To formulate the basic informations which could be applied to taxonomy of all members of the family Cyprinidae.

Chapter 2

Literature review

2.1 Taxonomy of *Puntius brevis, Systomus orphoides, Barbodes gonionotus* and *Hypsibarbus wetmorei* in Thailand

Thailand, which extends from latitude 5^0 45' to 20^0 30'N and from longitude $97^030'$ E to 105^0 45'E, approximately, 1,500 km from north to south and 800 km from east to west, covering 513,517 square km. The North lies between the Salween and Mekong River systems and most of the area is drained by the headwaters of the Chao Phraya River system. The central part has been intermingled by the canels of the Meklong, Chao Phaya and Bangpakong rivers.The Mekong system , including the Kwae Noi and Kwae Yai, originated from the Tennasserim range of the westernmost areas. The term Peninsula Thailand is used to refer to that area south of 120^0N latitude of southward from the Isthmus of Kra. The Salween system lies with the Indo-Burmese subregion, the Peninsula is at the extreme north of the Sundiac subregion, whereas the rest is belonged to the Indochinese subregion (Vidthayanon, Karnasuta and Nabhitabhata, 1997).

For the river systems of Thailand, in which were referred and were zoogeographically classified by Kottelat(1989) and Rainboth (1991) cited in Vidthayanon et al.(1997) which consist of six major river drainages (figure 3.1), there are the (1) Salween (Sw), (2)Mekong (MK), (3)rivers of southern (St) Peninsula, (4)south eastern (E) part, (5)Chao Phraya (CP) and (6)Meklong (Mkl) river systems. Above these river habitats, there are watershed and waterfalls.

According to the above river systems, four cyprinid fishes in this study have reported in all excepting for the Salween system. Moreover *Hypsibarbus wetmorei* also has never been found in the south east river system (Smith, 1945; Kottelat, 1989).

However, Magtoon and Donsakul (1990) collected *Systomus orphoides*' specimens from Pai River, one of the river in the Salween system.

According to Smith(1945)'s monograph which was the study of the fresh-water fishes of Thailand, fishes in the genus *Puntius* Hamilton,1822 comprise the most numerous species and among the most abundant as to individuals among the fresh-water fishes in Thailand. He described that it was convenient to adopt a section for Thailand barbs. Taxonomically, the generic name *Puntius*, originally named by Hamilton in 1822 to include a number of Indian species and later referred by Bleeker in 1863 who proposed three subgenera based on the number of barbels, namely, *Barbodes*, *Copoeta* and *Puntius* that had 4 barbels, 2 barbels and no barbels, respectively. Smith (1945) disagreed with Bleeker's idea and mentioned that *Barbodes* with 4 barbels should be properly placed within *Puntius*. Because the diagnosis of *Puntius* in this monograph was taken solely from Weber and de Beaufort(1916) 's work which was concerned largely on specimens from southeast Asia. From this work it happened that they did not try to make use the subgeneric groupings based on the number of barbels, although they did use the various possibilities in their key to species (Rainboth, 1996b). Therefore, Rainboth (1996b), who studied fishes of the Cambodian Mekong, has divided genus *Puntius* by Smith(1945) to four genera. They comprised *Puntius*, *Systomus*, *Barbodes* and *Hypsibarbus*. Their distinctive descriptions are given as follows.

Genus *Puntius* was described by having smooth dorsal-fin spine, 2 maxillary barbels(1 per side) and gill rakers 12 to 20 on first gill arch.

Genus *Systomus* was described by having finely serrated dorsal-fin spine, 2 or 4 barbels and gill rakers fewer than 12 on first gill arch.

Genus *Barbodes* was described by having serrated dorsal-fin spine, 8 branched pelvic-fin rays, skin of lower lip separated from lower jaw by a shallow groove and anal-fin base long 90% of head length and no tubercles on snout.

Genus *Hypsibarbus* was described by having serrated dorsal-fin spine, 8 branched pelvic-fin rays, skin of lower lip discontinuous with lower jaw which as separated by a shallow groove, anal-fin base 60% of head length and black scale margins gave a reticulated color pattern.

To certify Rainboth(1996b)' s idea of recognizing the mentioned genera at separated generic level. The following four species, each of which is representative species of each genus, are therefore selected to meet the objectives.

Family Cyprinidae

    Subfamily Cyprinina

        Tribe Systomini

            Subtribe Systomi

                Genus *Puntius*

                    Species *Puntius brevis*

                Genus *Systomus*

                    Species *Systomus orphoides*

            Subtribe Semiploti

                Genus *Barbodes*

                    Species *Barbodes gonionotus*

                Genus *Hypsibarbus*

                    Species *Hypsibarbus wetmorei*

2.2 Karyotypes and NOR phenotypes

Karyotypes defined as the chromosomal complement of a cell or organism (Quicke, 1993) which were visualized on a microscope then were photographed as they appeared spread out in a preparation (Halnan, 1989) and each organism' s karyotypes depended on size, centromere position on chromosome or chromosome type. To get chromosomes for karyotypes, it is necessary to inhibit cell cycle at metaphase so colchicine has been used. Colchicine has been a common use as mitotic inhibitor which

held metaphase throughout its activity and does not allow cell to enhance the next step of mitosis, anaphase, by unformation of the spindle which is an important unstable organelle in division's cell. Without the interference of colchicine to prevent the formation of microtubules of the spindle is easily occurred (Halnan, 1989). Colchicine is an alkaloid of *Colchicum autumnale*. This alkaloid has effected in cell by virtue of its ability to bind tubulin which is a monomer of the mitotic spindles and causes depolymerization and disappearence of the fibrillar microtubules (Insel, 1996). This is the reason why is widely used to be the mitotic inhibitor for getting metaphase chromosomes.

Besides the study of conventional karyotypes as above, chromosomes could be studied the other methods to get more details or more accuracy for matching homologous chromosomes. Banding techniques were used for these methods such as C-bands, G-bands, R-bands, Q-bands and NOR-bands. In this review, NORs were intended so that corresponded with this study.

NORs are thought to represent the chromosomal sites containing multiple copies of the 18S and 28S ribosomal RNA genes, rDNA, which presumably are actively transcribed at a preceding interphase (Gold et al., 1990; Pendas, Moran and Garcia-Vazquez, 1993). Cytological methods for detecting these sites on chromosomes use silver (Ag) staining remnants of the rRNA-protein complex synthesized by active genes prior to interphase (Fakan and Hernandez-Verdun, 1986, cited in Rab et al., 1996). It has been proposed that silver staining demonstrates some acidic nucleolus proteins that remain associated at the secondary constriction or the satellite of chromosomes but not the rDNA itself (Goodpasture and Bloom, 1975; Suja and Hernandez-Verdun, 1996).

Since comparative karyology has become a useful tool in fish systematic study (Buth et al., 1991 cited in Magtoon and Arai, 1993) and karyotype information effectively forms an independent data set for phylogenetic analysis and has probably been most useful in the investigation of groups of closely related and morphologically similar organisms(Quicke,1993). NOR phenotype which one type of cytotaxonomy can be utilized as characters for testing or inferring hypotheses of cyprinid relationship (Jenkin

et al., 1992) and displayed by the location, number and size of NORs (Rodrigues and Collares-Pereira, 1996).

There were documents that studied about NOR phenotypes in fish as follows:

Foresti, Almeida-Toledo and Toledo (1981) reported five species of the order Gymnotiformes from Brazil, comprised of *Gymnotus carapo*, *Apteronotus abifrons*, *Sternopygus macrurus*, *Eigenmannia virescens* and *Eigenmannia* sp. They found that all five species presented only one pair of homologs bearing NORs. In addition, Fernandes-Matioli ,Almeida-Toledo and Toledo-Filho(1997) reported extensive NOR polymorphism in six populations of *Gymnotus carapo* from the upper Parana river system and Brazil also.

Sanchez et al.(1990) analyzed the structure and variability of NORs of brown trouts (*Salmo trutta*) by C- , Ag- and restriction endonuclease banding 's using which revealed great variability in the size of the short arm of the NOR-bearing chromosome.

Magtoon et al.(1992) examined karyotype evolution including NORs and geographical distribution of the Thai medaka (*Oryzias minutillus*) in 18 localities within Thailand. In this paper, they described karyotypes and NOR polymorphism of *Oryzias minutillus* from each localities and discussed the relationship between karyotype evolution and geographical distribution of this species.

Pendas et al. (1993) reported that rRNA genes were interspersed throughout a heterochromatic chromosome arm in Atlantic salmon (*Salmo salar*) by using four rDNA probes of fluorescent *in situ* hybridization (FISH) including NORs' detection.

Vitturi et al.(1996) described characterization of mitotic chromosomes ,karyotypes and chromosomal phenotypes , and they reported the finding of intraheteromorphism and interspecific variability in the number and the position of NOR-bearing chromosomes of four Mediterranean species of the genus *Diplodus*.

Zhang and Tiersch (1997) studied chromosomal inheritance patterns of intergeneric hybrids of Ictalurid catfishes of which having three species viz., *Ictalurus punctatus, Ameiurus melas* and *Pylodictis olivaris*, and two hybrids, *Ictalurus punctatus* x *Ameiurus melas, Ictalurus punctatus* x *Pylodictis olivaris*, by analysis of chromosome formula and NOR phenotypes.

In the family Cyprinidae, Buth et al. (1991) cited in Rab, Karakousis et al.(1996) reviewed that more than 15 different NOR phenotypes have been discovered among the 100+ species of karyotypically conservative north American minnows. Similar interspecific NOR variation have been discovered among 30+ species of Japanese cyprinids (Takai and Ojima, 1992 cited in Rab, Karakousis et al., 1996). While Eurasian leuciscine cyprinids, cyprinid fishes in genus *Leuciscus* from both Europe and Asia,seem to be less variable although data on NOR phenotypes are available for only some10+species (Rab and Collares-Pereira,1995). However, African cyprinids seem to be no data on NOR phenotypes, except conventional karyotypes of *Barbus* sp. (Rab et al., 1995). Because of its large group and great distribution, there were other data about cyprinids' NOR phenotypes as follows.

Mayr, Rab and Kalat (1986) investigated comparative cytogenetic determination of the number and localization of Ag-NOR-positive chromosomes of seven cyprinid species, they found three species in dipliod (*Scardinius erythrophthalmus, Leucaspius delineatus* and *Tinca tinca*) and four species in polyploid(*Cyprinus carpio, Carassius carassius, Carassius auratus gibelio* and *Carassius auratus auratus*), together with investigation in the genomes of the mentioned species by counterstain-enhanced fluorescence methods.

Amemiya and Gold (1988) reported substantial interspecific variation in chromosomal NORs among ten cyprinid fishes in north America, the southern United States, this variation was taxonomically informative.

Furthermore, the chromosomal NOR database appeared to provide a framework of which systematic or phylogenetic inferences could be made. Therefore, Jenkin et al. (1992) and Powers and Gold (1992) focused to cytogenetic studies in North American Minnows. The first group studied chromosomal NOR phenotypes among 21 species and proposed the relationships among these cyprinids. For the second group, they examined chromosomal NOR variation in the 11 species of *Luxilus*.

Almeida-Toledo et al.(1995) studied chromosomal location of NORs and C-bands in F$_1$ hybrid of bighead carp (*Aristichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*) that reared in Brazil. They found that C-patterns were distinctive. In contrast, NORs pattern were ineffective in hybrids of both *A.nobilis* and *H.molitrix*, they, however, show multiple NORs located in the same kind of chromosomes and at the same positions.

Rab, Karakousis et al.(1996) described banded karyotype of the cyprinid fish , *Leuciscus borysthenicus*, from the Strymon River, Greece. This study described karyotype, C-banding and NOR band. For NOR band, they discussed location of NORs which has one pair on subtelocentric chromosomes' short arms.

Rodrigues and Collares-Pereira (1996) examined chromosomal polymorphism of Iberian species (*Chondrostoma lusitanicum*) from Portugal by using C-banding, silver-staining and fluorescent staining with chromomycin A$_3$ (CMA$_3$). For NOR polymorphism, they described variability in the number of NOR regions per genome, number of active NOR site per cell and relative size of individual NORs. While Collares-Pereira et al. (1998) studied two leuciscine species, *Leuciscus carolitertii* and *Leuciscus pyrenaicus* also from Portugal. The work cytogenetically studied NORs. For both species, their NORs apparently located in one small submetacentric pair of chromosomes.

For cyprinid fishes in Thailand, chromosome data were mostly informed in terms of conventional karyotypes (Magtoon and Arai ,1989; Magtoon and Donsakul , 1990). There was only Magtoon and Arai (1993) who studied NORs' distribution of four cyprinid

fishes, *Cirrhinus jullieni*, *Osteochilus waanders*, *Cyclocheilichthys enoplos* and exotic *Labeo rohita*, including is also their study of karyotypes of the species.

Chapter 3

Materials and Methods

3.1 Locality: The Chao Phraya River System

Van Beek (1995) was cited in Vidthayanon et al. (1997), he described this river system as is seemed to be the main blood vessels of the northern and central lower plain of Thailand, originated from watersheds of the two mountainous ranges, Thanonthongchai and Peepunnum Ranges. The watershed systems have been drained into by four major rivers; Ping, Wang, Yom and Nan Rivers, conjuncted into mainstream of Chao Phraya River at Pak Nampo, Nakonsawan province northern Central Thailand. The river connects with three tributaries; Sakaekrung and Tachin from western, Pasak from the Petchabun Range, northern and Bangpakong from the Bantad range, southeastern, then the system run off to the gulf of Thailand. Historically, The central lower plain had raised above the sea level during 1000-5000 years ago.

3.2 Specimens

Specimens in this study were collected by seine from natural water way, between March 1998 to December 1999 in the central plain, *Puntius brevis* and *Systomus orphoides'* specimens were captured from Amphur Tha Wung, Lop buri and for *Barbodes gonionotus* and *Hypsibarbus wetmorei* they were captured from Amphur Phayuha Khiri, Nakhon Sawan, respectively. Specimens were kept alive, brought back, maintained in well-aerated aquaria at the laboratory at the Biology Department, Faculty of Science, Srinakharinwirot University at Prasanmitr, Bangkok. Their localities were approximately shown in figure 3.1 with details in figure 3.2.
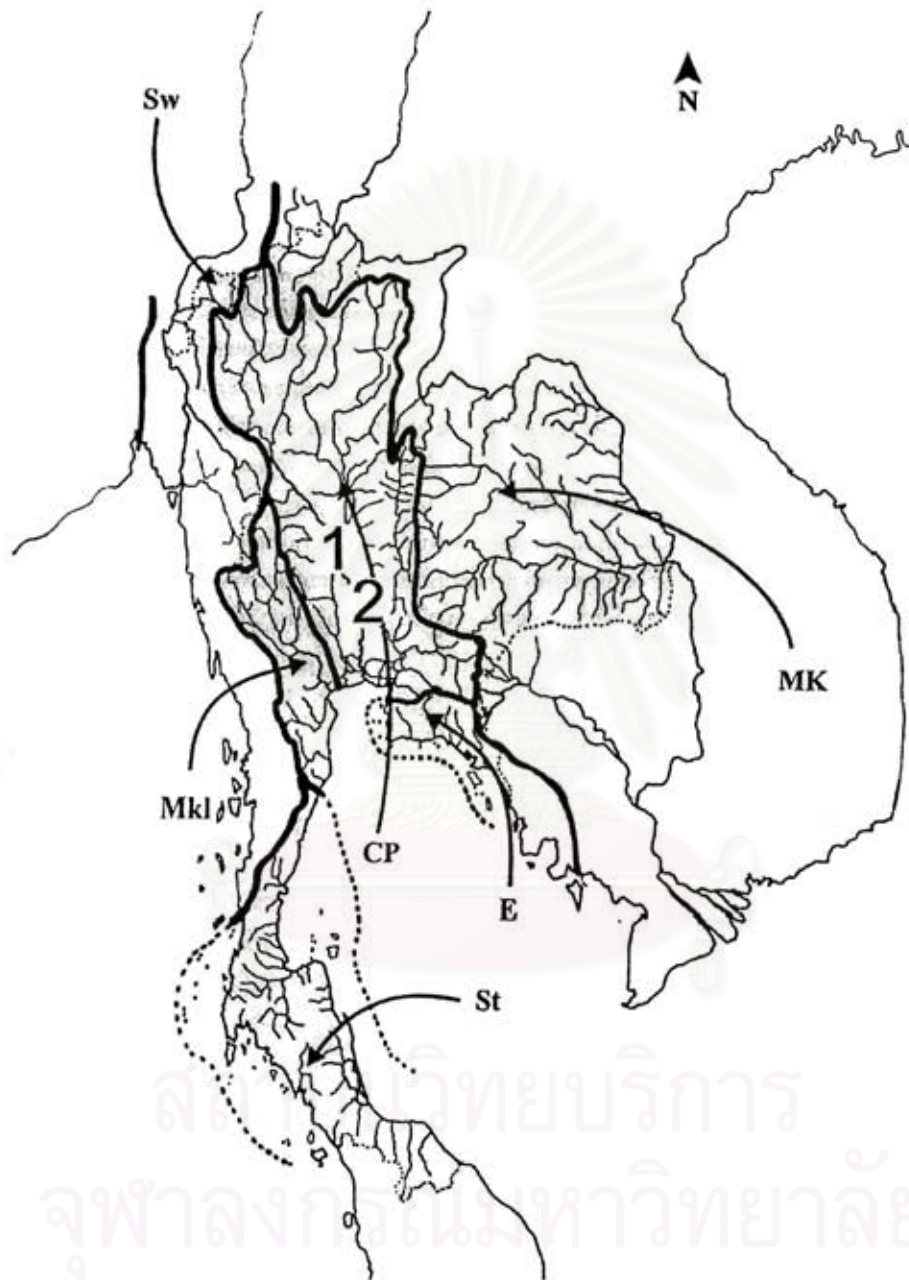
Figure 3.1 Map of Thailand, to show localities of sampling specimens; numbers indicated species (1 represented *Hypsibarbus wetmorei* and *Barbodes gonionotus* and 2 represented *Systomus orphoides* and *Puntius brevis*) modified from Vidthayanon et al. (1997).
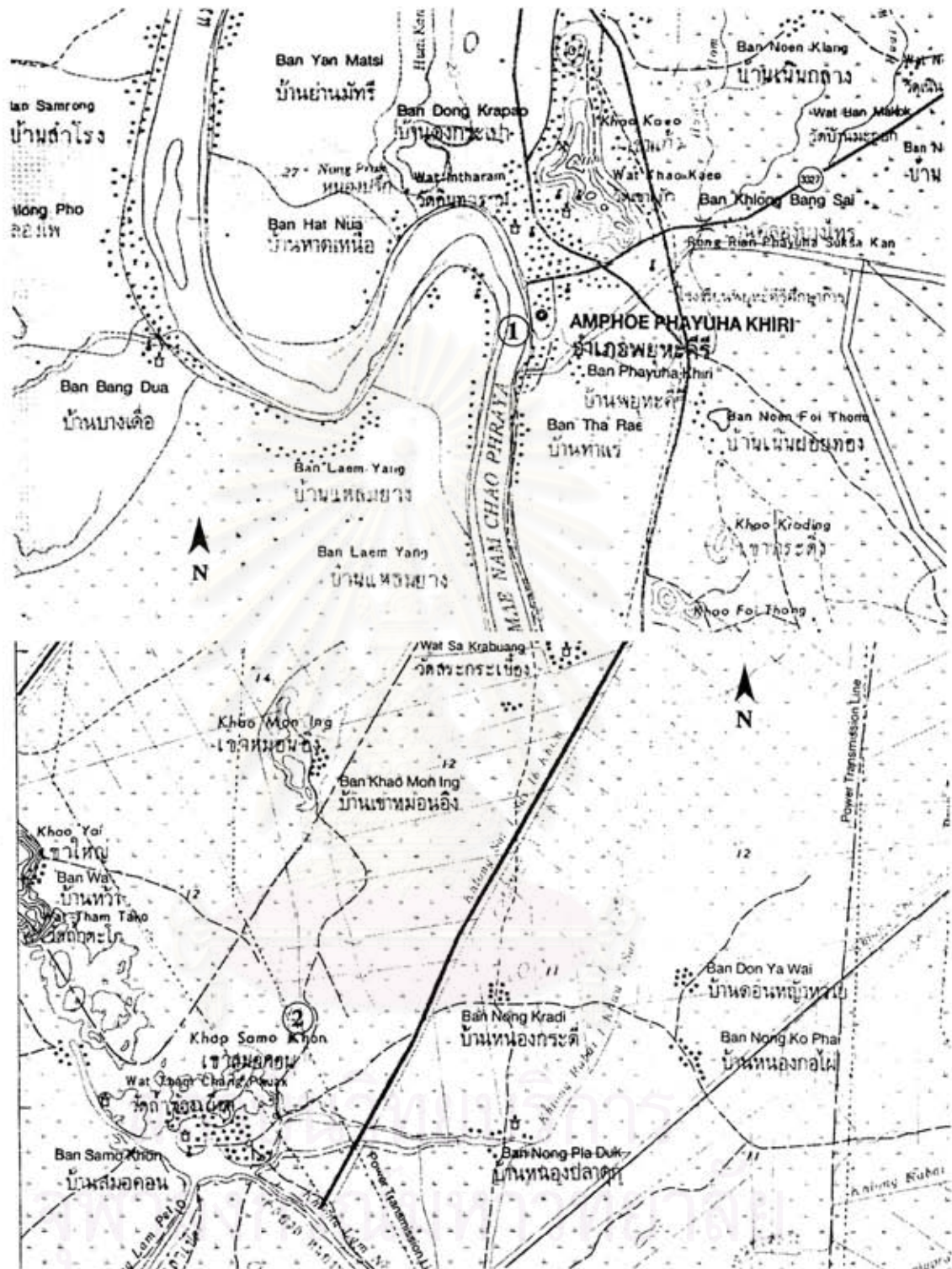
Figure 3.2 Map of collecting localities of specimens(1) located in the Chao Phraya river between 15^0 27'-28' N and $100^0$8'-9'E and specimen (2) located the paddy field between $14^0$54'-55' N and $100^0$31'-32' E which modified from Royal Thai Survey Department (map, 1994) with 1:50,000 scale.

3.3 Morphometric study

10 individuals of each species apart from living specimens were preserved in 70% ethanol for morphometric measurements and meristic counts. The methods were drawn from Lagler et al.(1977) the followings with some modifications were subjects of the present study:-

Total length(TL)
Standard length(SL)
Body depth(BD)
Head length(HL)
Snout length(STL)
Eye diameter(EL)
Postorbital length(PL)
Dorsal fin rays' count
Anal fin rays' count
Pelvic fin rays' count
Pectoral fin rays' count

3.4 Karyotypes' study

In this study, metaphase chromosomes will directly obtain from gill or kidney, whatever be suitable condition. As a reason to use them for metaphase preparation because they were all good sources for actively dividing cells while kidney in most fishes contains the hematopoietic organs in the renal intertubular tissue and gill has epithelial cells (Gold et al., 1990).

Chromosomes of this study were obtained directly from live specimens by method of which modified from Kligerman and Bloom (1977); Gold (1984); Gold et al. (1990); Foresti, Oliveira and Almeida-Toledo (1993) and was taken step by step as the followings:

3.4.1 Colchicine treatment

Live specimen was injected predorsally with 0.1 %W/V of colchicine in which quantity was equaled to 0.2 cc./individual 'standard length and then returned to aerated aquarium for 4-6 hours before sacrifice on ice.

3.4.2 Tissue preparation

As soon as fish stayed still on ice, tissue from the gills was taken, by opening the operculum and pulling a forceps over the filaments of the branchial arches; or the kidney by dissecting out pieces of cephalic kidney. Tissues were washed 2-3 times in a Petri dish at room temperature containing distilled water to remove any adhering fat, blood or debris.

3.4.3 Hypotonic treatment

Placed tissues into about 10 times their volume of 0.057% KCl hypotonic solution and finely minced tissues in Petri dish, then transferred this suspension to the centrifuged tube for 45 minutes at 4^0C and brought this tube to be centrifuged at 1,000 rpm for 5 minutes.

3.4.4 Fixation procedure

Discarded the supernatant and added freshly prepared fixative (3 ethanol: 1 acetic acid) including gently stirring with a Pasteur pipette. Stored for at least 25 minutes at room temperature then centrifuged at 1,000 rpm for 5 minutes. Discarded the supernatant and repeated this procedure at least twice.

3.4.5 Slide preparation

Dropped 2 drops of the concentrated suspension onto a new and clean slide by a Pasteur pipette at room temperature and tilted and let it be truly dry. Repeated this procedure to other slides. For the remained suspension in fixative, tightly closed the tube with parafilm and stored in the refrigerator at most 1 month.

3.4.6 Staining chromosomes

Stained slides in 5% Giemsa' s working solution which prepared from 1 part of Giemsa' stock solution and 20 parts of Sorensen' s phosphate buffer pH 6.8 (one part of 2.75% Na_2HPO_4 solution and one part of 2.84% KH_2PO_4 solution) for 2 hours. Rinsed briefly in distilled water and let them dried in air. Observed chromosomes under the light microscope and marked the position of the chromosomes which were good enough for counting, karyotypes and NOR banding' s analysis in order to taking a photomicrograph.

3.4.7 Chromosome photomicrography

Recorded the chromosomes by Olympus' camera, PM 35-AD type, which connected with Olympus' light microscope, BHA type, which used 100 times of objective lens and 5 times of eye lens. This instrument was automatically in controlling. Photomicrography of appropriate spreads was carried out by using Kodak technical Pan 2415 film (ASA 100) developed in HC-110(Dil B) which was Kodak' s trademark.

3.5 NOR phenotypes' study

In order to use chromosome number from conventional karyotypes more meaningfully, it is necessary to be able to identify individual chromosomes (Quicke, 1993). In addition, banding techniques, especially NOR-bands, have been useful as stated formerly. Hence, NORs of the four studied species will be examined in addition to their karyotypes.

Chromosomes of this stage came from above preparation of Giemsa' s staining. Each which contained chromosomes for silver staining, needed to destain in absolute ethanol for 5 minutes and air-dried. After air-drying, the result was ready to stain with silver. Silver staining method was that of Howell and Black (1980) and Gold and Ellison (1982) which was remarked as one-step silver staining method. The procedure was as follows:

- Placed 2 drops of the gelatin solution, 2 g powdered gelatin USP in 100 ml deionized water and 10 ml pure (91.5 %) formic acid, and 4 drops of the $AgNO_3$ solution (50%W/V) on a slide. Mixed the solutions gently by tilting the slide from side to side. Covered with a cover glass.

- Kept the slide on a slide warmer set to 40-45^{0}C until the solution turns golden brown (approximately 6-8 minutes).

- Removed the cover glass and rinsed the slide thoroughly in distilled water to remove cover glass. Fixed in 5% sodium thiosulfate for 4-5 minutes and rinsed briefly in distilled water again.

- Counterstained with 5% Giemsa solution, rinsed briefly in distilled water and let it dried in air.

- Cleared in xylene for 10 minutes and mounted in permount.

- Recorded the chromosomes.

3.6 Chromosomal analysis

For the conventional karyotypes, the frequency for each chromosome count was observed and the principal count was taken as the diploid number for each species. Five best metaphase spreads for each species were measured in comparison with a stage micrometer in order that constructed the idiogram of each species. Chromosomes from these spreads were classified according to the criteria nomenclature of Levan, Fredga and Sandberg (1964) as shown in table 3, and calculated fundamental number or arm number (NF) which metacentrics(m) and submetacentrics(sm) were described as two-arm chromosomes, whereas subtelocentrics (st)and acrocentrics(a) as one-arm chromosomes.

Table 3 Classification of chromosomes and their arm ratio

Chromosomal type	Arm ratio*
Metacentrics (m)	1.0-1.7
Submetacentrics (sm)	>1.7-3.0
Subtelocentrics (st)	>3.0-7.0
Acrocentrics (a)	>7.0

* arm ratio(r) defined as long arm(l) divided by short arm(s) so this mathematical equation is r= l/s.

For NOR phenotype analysis, NOR stained metaphase plates which NOR regions on chromosomes should be stained black, compared with original-metaphase plates in order to identifying the position of the NOR on the chromosome and the centromere position of homologous chromosome.

Chapter 4

Results and Discussion

4.1 Description of species(morphometric, meristic)

Scientific name: *Puntius brevis* (Bleeker, 1860)

Thai name: pla tapien sai

Common name: golden little barb

Figure 4.1 *Puntius brevis* (Bleeker, 1860)

Symnonyms: *Puntius puntio* Hamilton, 1822

Capoeta javanicus Bleeker, 1850

Systomus (Capoeta) brevis Bleeker,1860

Systomus (Capoeta) leiacanthus Bleeker,1860

Puntius (Capoeta) brevis Bleeker, 1863

Capoeta brevis Bleeker, 1863

Puntius (Capoeta) leiacanthus Bleeker, 1863

Barbus leiacanthus Günther, 1868

Barbus brevis Günther, 1868

Barbus sophoroides Günther, 1868

Puntius sophoroides Smith, 1945

References: Smith(1945), Taki(1974), Rainboth(1989), Jayaram(1991), Roberts and Warren(1994), and Rainboth(1996b).

Diagnosis: A *Puntius* species differing from other related *Puntius* species by having no discrete dark spot at base of anterior dorsal-fin rays and having complete lateral line (Rainboth, 1996b).

Morphometric and meristic data based on 10 individuals as shown in tables 4.1 and 4.2, respectively.

Body compressed and oblong, body depth 42.30% in standard length. Head length 27.74% in standard length, head slightly more compressed than body with short maxillary barbels. Snout shorter than eye diameter. Mouth terminal. Body covered with cycloid scales, a dark spot at caudal peduncle. Lateral line complete. Caudal fin forked.

Dorsal fin ray 9, first spine simple with no serration, second to ninth rays branched. Membrane of dorsal fin usually with dark stripes. Anal fin rays 6, first spine simple, second to sixth rays branched. Pectoral fin rays 10-12 (usually 11), pelvic fin rays 8-9 (usually 9).

Scientific name: *Systomus orphoides*(Valenciennes in Cuvier and Valenciennes, 1842)

Thai name: pla kam cham

Common name: red-cheek barb

Figure 4.2 *Systomus orphoides*(Valenciennes in Cuvier and Valenciennes, 1842)

Symnonyms: *Barbus rubripinna* van Hasselt, 1823

Barbus gardonides Valenciennes in Cuvier and Valenciennes, 1842

Barbus orphoides Valenciennes in Cuvier and Valenciennes, 1842

Barbus sarananella Bleeker, 1850

Systomus (Barbodes) rubripinnis Bleeker, 1860

Puntius caudimarginatus Blyth, 1860

Puntius (Barbodes) rubripinna Bleeker, 1863

Barbus rubripinnis Peters, 1868

Barbus rubripinnis Günther, 1868

Barbodes rubripinnis Jordan and Seale, 1908

Puntius sarana Smith, 1945

Puntius simus Smith, 1945

Puntius jacobusboehlkei Fowler, 1958

References: Smith(1945), Taki(1974), Rainboth(1989), Jayaram(1991), Roberts and Warren(1994), and Rainboth (1996b).

Diagnosis: A *Systomus* species differing from other related *Systomus* species by having anterior maxillary barbels, 16 scale rows around caudal peduncle and having very brightly color, bright red tail bordering by black submarginal stripes(Rainboth, 1996b).

Morphometric and meristic data based on 10 individuals as shown in tables 4.1 and 4.2,respectively.

Body compressed and oblong, body depth 37.52% in standard length. Head length 25.93% in standard length, head slightly less compressed than body with 4 barbels. Snout shorter than eye diameter. Mouth terminal. Body covered with cycloid scales and had a dark spot on caudal peduncle. Body with pale color or colorless when captured. Lateral line complete. Caudal fin forked.

Dorsal fin ray 9, first spine simple with serration, second to ninth rays branched. Anal fin rays 6, first spine simple, second to sixth rays branched. Pectoral fin rays 12-13 (usually 13), pelvic fin rays 8-9 (usually 9).

Scientific name: *Barbodes gonionotus* (Bleeker, 1850)

Thai name: pla tapien khao

Common name: common silver barb

Figure 4.3 *Barbodes gonionotus* (Bleeker, 1850)

Symnonyms: *Barbus gonionotus* Bleeker, 1850

Puntius (Barbodes) javanicus Bleeker, 1865

Barbus (Puntius)javanicus von Martens, 1876

Puntius javanicus Sauvage, 1881

Barbus (Puntius) javanicus Hora, 1923

Puntius (Barbodes)jolamarki Smith, 1934

Barbus jalamarki Fowler, 1937

Puntius viehoeveri Fowler, 1943

Puntius gonionotus Smith, 1945

References: Smith(1945), Taki(1974), Rainboth(1989), Jayaram(1991), Roberts and Warren(1994), and Rainboth(1996b).

Diagnosis: A *Barbodes* species differing from other related *Barbodes* species by having slate-grey caudal fin, 6-7 branched anal-fin rays and no red coloration on body or fins (Rainboth, 1996b).

Morphometric and meristic data based on 10 individuals as shown in tables 4.1 and 4.2,respectively.

Body compressed and oblong, body depth 40.27% in standard length. Head length 26.33% in standard length, head slightly less compressed than body with 4 barbels. Snout shorter than eye diameter. Mouth terminal. Body covered with cycloid scales. Lateral line complete. Caudal fin forked.

Dorsal fin ray 9, first spine simple with serration, second to ninth rays branched. Anal fin rays 7, first spine simple, second to seventh rays branched. Pectoral fin rays 12, pelvic fin rays 8-9 (usually 9).

Scientific name: *Hypsibarbus wetmorei* (Smith, 1931)

Thai name: pla tapak

Common name: golden belly barb
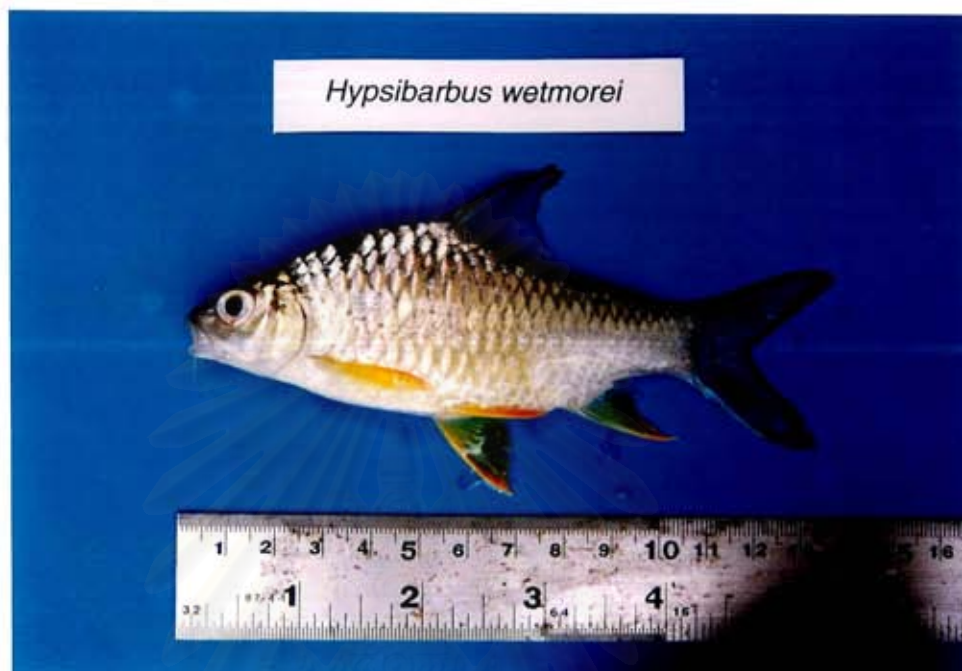
Figure 4.4 *Hypsibarbus wetmorei* (Smith, 1931)

Symnonyms: *Barbus goniosoma* Duncker (not Bleeker), 1904

                  Puntius wetmorei Smith, 1931

                  Puntius (barbodes) daruphani Smith, 1934

                  Barbus daruphani Fowler, 1937

                  Puntius daruphani Smith, 1945

                  Barbus beasleyi Smith, 1945

                  Puntius daruphani tweediei Menon, 1954

                  Lissochilus annamensis Mai (not Pellrgrin and Chevey), 1978

                  Hypselobarbus cf. daruphani Roberts and Warren, 1994

References: Smith(1945), Taki(1974), Rainboth(1989), Jayaram(1991), Roberts and Warren (1994) and Rainboth(1996a,b).

Diagnosis: A *Hypsibarbus* species differing from other related *Hypsibarbus* species by having red to reddish-orange pelvic and anal fins, robust body (not compressed), 3-5 gill rakers on lower arm of first arch, 7-9 (mean 8.3) predorsal scales and 9-14 (mean 11.7) dorsal-spine serrations(Rainboth, 1996b).

Morphometric and meristic data based on 10 individuals as shown in tables 4.1 and 4.2,respectively.

Body compressed and oblong, body depth 41.80% in standard length. Head length 23.56% in standard length, head slightly less compressed than body, with 4 barbels. Snout shorter than eye diameter. Mouth terminal. Body covered with cycloid scales and with reddish-orange color on belly area. Lateral line complete. Caudal fin forked.

Dorsal fin ray 9, first spine simple with serration and second to ninth rays branched. Anal fin rays 6, first spine simple, second to sixth rays branched. Pectoral fin rays 12, pelvic fin rays 9.
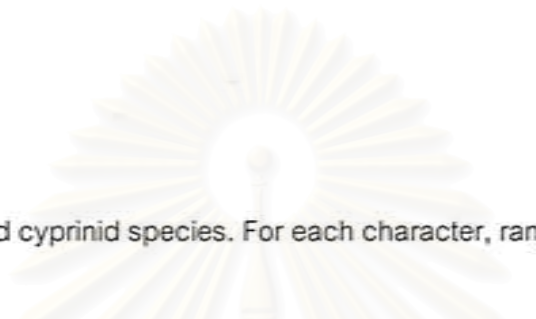
Table 4.1 Morphometric study for the four studied cyprinid species. For each character, range and mean ± SD are indicated.

Characters	Puntius brevis 10 specimens		Systomus orphoides 10 specimens		Barbodes gonionotus 10 specimens		Hypsibarbus wetmorei 10 specimens	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Total length(mm)	56-81	70.9 ± 7.65	87-119	100.2 ± 10.68	103-137	126.2 ± 9.81	114-158	135.4 ± 15.72
Standard length(mm)	49-69	61.5 ± 5.91	77-99	88.9 ± 7.96	88-119	109.5 ± 9.62	94-140	115.9 ± 14.60
Body depth(mm)	21-30	26 ± 3.06	27-37	33.3 ± 2.75	32-49	44.1 ± 5.34	37-58	48.4 ± 6.02
Head length(mm)	13-19	17.05 ± 1.64	21-26	23.0 ± 1.63	26-31	28.75 ± 1.93	22-31	27.3 ± 3.53
Snout length(mm)	2.15-4.55	3.38 ± 0.67	3.9-6.45	4.86 ± 0.83	2.85-7.45	5.08 ± 1.21	4.25-7.95	6.26 ± 1.24
Eye diameter(mm)	3.9-6.8	5.34 ± 0.75	5.5-6.95	6.24 ± 0.53	8.2-11.5	9.42 ± 1.04	7.85-11.7	9.37 ± 1.31
Postorbital length(mm)	5.25-8.55	7.16 ± 0.91	9.9-19	12.81 ± 3.27	12.05-13.5	12.94 ± 0.48	9.6-13.5	11.96 ± 1.34

Table 4.2 Meristic counts for the four studied cyprinid species. For each character, range and mode (in parentheses) are indicated.

Characters	Puntius brevis 10 specimens	Systomus orphoides 10 specimens	Barbodes gonionotus 10 specimens	Hypsibarbus wetmorei 10 specimens
Dorsal fin rays	9	9	9	9
Anal fin rays	6	6	7	6
Pelvic fin rays	9	9	8-9(9)	9
Pectoral fin rays	10-12 (11)	12-13(13)	11-12(12)	12

4.2 Karyotypes and Chromosomal NOR- phenotypes

4.2.1 Frequency observation of chromosome diploid number

From chromosome counts of four studied species, all of them had the diploid number from mode in each species which equaled to 50 as table 4.3. As same as results from Magtoon and Arai(1989) and Magtoon and Donsakul(1990) also.

Table 4.3 Frequency distributions of diploid chromosome counts.

Species	Examined specimens	2n									Total counts
		43	44	45	46	47	48	49	50	51	
Puntius brevis	8	1	1	-	4	1	3	5	112	1	128
Systomus orphoides	6	-	-	-	-	-	2	6	114	-	122
Barbodes gonionotus	10	-	-	-	2	-	2	6	138	-	148
Hypsibarbus wetmorei	10	-	1	1	1	1	2	4	111	-	121

4.2.2 Description of karyotypes and NOR-phenotypes

Puntius brevis

Based on 112 metaphase counts per 8 specimens, the diploid number was 2n= 50. The karyotype comprised of 2 metacentric chromosomes, 2 submetacentric chromosomes, 2 subtelocentric chromosomes and 44 acrocentric chromosome (figure 4.5 and 4.6). The arm number (NF) was 54. The average chromosome length range from 5 metaphase plates was 1.88- 4.01 μm. Nucleolus organizer regions (NORs) were located on the satellited regions of a subtelocentric pair on short arms(table 4.4).

Systomus orphoides

Based on 114 metaphase counts per 6 specimens, the diploid number was 2n= 50. The karyotype comprised of 12 metacentric chromosomes, 20 submetacentric

chromosomes, 4 subtelocentric chromosomes and 14 acrocentric chromosome (figure 4.7 and 4.8). The arm number was 82. The average chromosome length range from 5 metaphase plates was 1.49- 2.72 µm.

NORs were located on the satellited regions of a submetacentric pair on short arms (table 4.4).

Barbodes gonionotus

Based on 138 metaphase counts per 10 specimens, the diploid number was 2n= 50. The karyotype comprised of 6 metacentric chromosomes, 16 submetacentric chromosomes, 6 subtelocentric chromosomes and 22 acrocentric chromosome (figure 4.9 and 4.10). The arm number was 72. The average chromosome length range from 5 metaphase plates was 2.12-4.31 µm. NORs were located on the satellited regions of a submetacentric pair on short arms and on the satellited regions of an acrocentric pair on short arms(table 4.4).

Hypsibarbus wetmorei

Based on 111 metaphased counts per 10 specimens, the diploid number was 2n= 50. The karyotype comprised of 12 metacentric chromosomes, 12 submetacentric chromosomes, 4 subtelocentric chromosomes and 22 acrocentric chromosome (figure 4.11 and 4.12). The arm number was 74. The average chromosome length range from 5 metaphase plates was 1.44- 3.27 µm. NORs were located on the satellited regions of an acrocentric chromosome pair on long arms(table 4.4).

For karyotypes, Magtoon and Arai(1989) who studied six cyprinid fishes from Ayuthaya reported that Puntius brevis had the arm number was 54(2m+2sm+46a), Barbodes gonionotus had the arm number was 72(2m+20sm+4st+24a) and Hypsibarbus wetmorei had the arm number was 70 (12m+8sm+6st+24a), respectively. Whereas Taki and Suzuki (1977) cited in Magtoon and Arai (1989) reported Systomus orphoides which was Indian and Ceylonese species had the arm number was 92 and Magtoon and Donsakul(1990) who studied three cyprinid fishes in Pai river of the

Salween river system reported *Systomus orphoides* had the arm number was 80 (14m+16sm+4st+16a), respectively, compared with this study they were different in arm numbers or chromosome types in spite of they were the same species. Donsakul (personal communication, 1999) explained that they might be happened if a study took place at a different time or a different worker which connected with chromosome measurement. Chromosomes might be measured different workers which had a different criteria to specify the centromeric position. Moreover, each cell does not have cell cycle to divide altogether such as chromosomes in mid-metaphase have less condensation than late metaphase. It might be that differences in chromosome fixation and preparation techniques could cause differential contractions of chromosomes and thereby change the relative number of chromosome types.

It is possible that some of the intraspecies karyotype differences seen in comparisons between different worker result from genetically isolated populations from different geographical regions. There were some evidences for these events in mitotic chromosomes of *Serrasalmus spilopleura* which were sampled from the Parana Paraguay rivers (Cestari and Galetti,Jr, 1992) ,*Oryzias minutillus* which were examined from different localities in Thailand (Magtoon et al., 1992)and *Gymnotus carapo* from six localities which were investigated from the Upper Parana river system (Fernandes-Matioli et al.,1997), including *Sternopygus macrurus* which had a different NOR phenotype in 3 Brazilian river basin (Dealmeidatoledo et al.,1993) also. Therefore each studied species was sampled from the same locality for each species to be make sure that there was no differences in intraspecies karyotypes of genetically isolated populations.

In this study, this was the first time for idiogram construction for four cyprinid fishes. Five metaphase plates per species which were used to measure for karyotype and idiogram construction were good spreading, not overlapping or clumping, besides they must be not too condensed to be difficult for measurement also.

For karyotype construction, although all of karyotypes were demonstrated have all the chromosome paired, it was realized that in some cases the pairing was arbitrary because of the similarity in size which some chromosomes of each species were so close in their length.

For comparison of four studied species' karyotypes, it can be seen that the relative numbers in chromosome types of metacentrics, submetacentrics, subtelocentrics and acrocentrics vary among species. Interspecies karytotype differences may be divided two groups between *Puntius brevis* and three other species which *Puntius brevis* had NF = 54 while three other species had more than 70. This opinion corresponded with Magtoon and Arai (1989)'s discussion also. However, the analysis from only conventional karyotype may not be truly enough, it is essential to conduct other studies, such as morphological characters, isozymes, DNA content or banding karyotypes, and organizes these informations for analysis.

For NOR phenotypes, it was clear that the number and the location of NORs on the chromosomes of each studied species were different. This NORs study was reported here for the first time of the old genus *Puntius* and because these cyprinid fishes had never been reported, a phylogenetic analysis of four studied cyprinid fishes can not be happened in this analysis.

From the prior results, interspecies heteromorphism observed in this study (table 4.4) comprised of the number of silver stained NORs and the chromosomal location of homologous chromosomes. Whereas NORs of four studied species were different in their own. In fact, *Puntius brevis* was reported silver-stained NORs on the subtelocentric chromosome pair but these chromosomes in some cases belonged to the submetacentric chromosome pair by measurement which resulted from less condensation (longer chromosomes). However, it was necessary to allow this pair was subtelocentric chromosomes from this disparity because every silver stained plates which worked out and five metaphase plates which were measured for karyotype and

idiogram construction were subtelocentric chromosomes by measurement. It is known that regions where the chromatid fiber failed to undergo coiling result in the appearance of centromere and nucleolus organizer (Ratter, 1992) which make them to be difficult to stain with Giemsa solution and to see with the light microscope. From this controversial result in another side, it was possible that *Systomus orphoides* and *Puntius brevis* may have a common in NORs.

Besides interspecies heteromorphism in NORs, there was intraspecies heteromorphism in individual specimen of each species even individual cell which efiect on a limitation of these interpretations. However, the specificity of chromosomal silver staining were at NOR sequences of rDNA that were actively transcribed at the preceding interphase and not in the sequences themselves which has been proposed that silver staining associated with some acidic nucleolus proteins at NOR sequences (Goodpasture and Bloom, 1975;Suja and Hernandez-Verdun, 1996) and might reflect rDNA' s level of transcriptional activity during this interphase also (Gerbi, 1997). Thus an important question to solve in the future was whether the NOR heteromorphisms represent loss (or gain) of their transcriptional activities loss (or gain) of NOR sequences themselves. This question can be directly proved by *in situ* autoradiography of chromosomes by using radioactively labeled rRNA or complementary DNA sequences (Gold, 1984) or the alternative technique is truly favorite and reliable at recent which is fluorescence *in situ* hybriization (FISH) (Rab et al., 1996).

In the future, if the NOR study of this fish group extended, NOR database might provide to help this controversial systematics. It is essential that this studied extension should be continued.

Table 4.4 Summary of cytogenetic data for the four studied cyprinid species.

Species	Karyotypes	and	Idiograms			NOR	Phenotypes		
	specimens	plates	NF[1]	ALR[2] (μm)	formula	specimens	plates	NOR pair	Formula[3]
Puntius brevis	5	5	54	1.88-4.01	2m+2sm+2st+44a	5	10	1	CC
Systomus orphoides	5	5	82	1.49-2.72	12m+20sm+4st+14a	3	7	1	DD
Barbodes gonionotus	5	5	72	2.12-4.31	6m+16sm+6st+22a	4	9	2	AA,DD
Hypsibarbus wetmorei	5	5	74	1.44-3.27	12m+12sm+4st+22a	5	10	1	BB

1, NF: arm numbers

2, ALR: average length range

3, formula (modified from Amemiya and Gold, 1988): A, terminal on short arms of acrocentric chromosome; B, terminal on long arms of acrocentric chromosome; C, terminal on short arms of subtelocentric chromosome; D, terminal on short arms of submetacentric chromosome
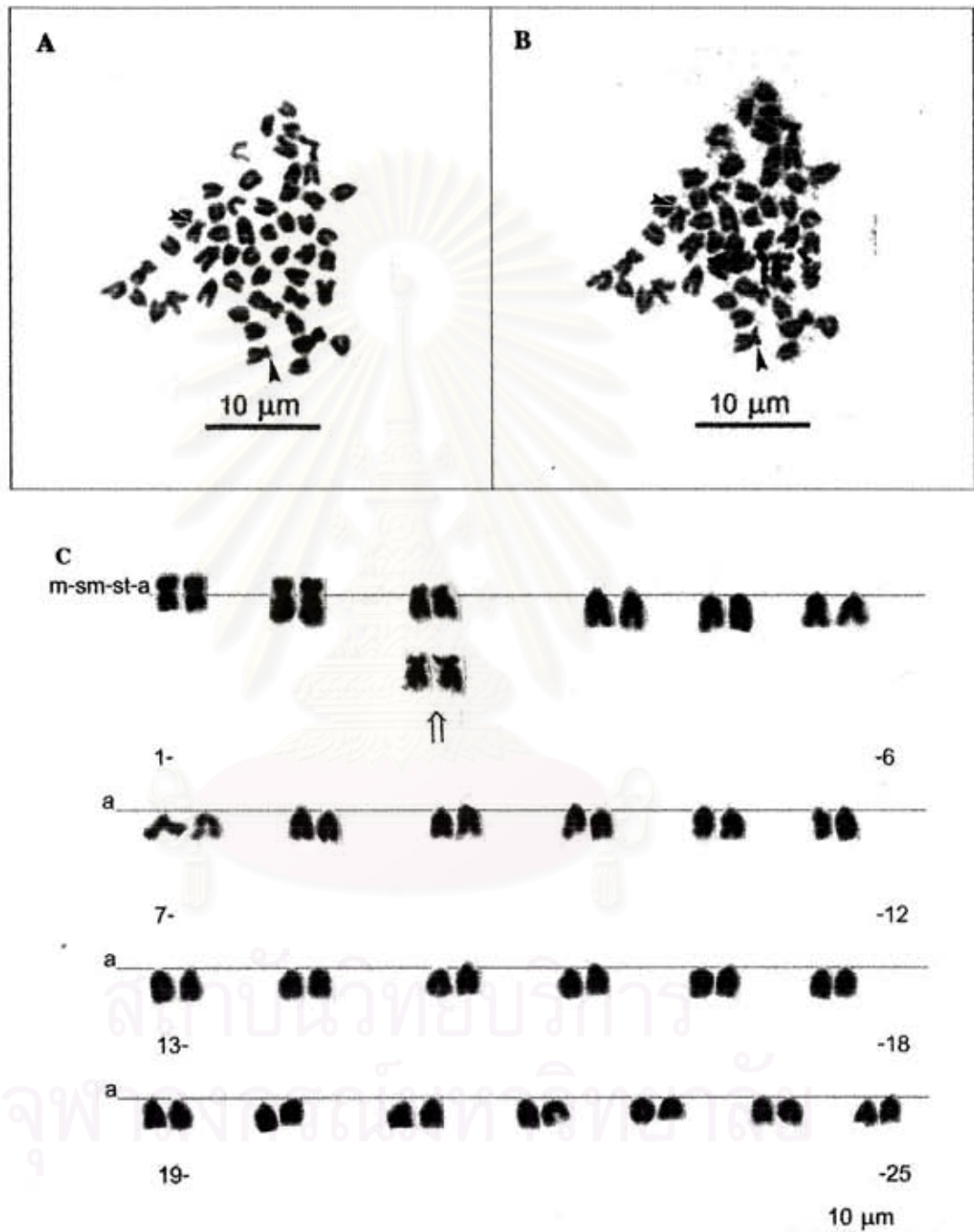
Figure 4.5 A. conventional Giemsa stained metaphase plate ,B. sequentially silver-stained metaphase plate and C. corresponding karyotype of Puntius brevis ; NOR bearing chromosomes indicated by arrow heads A. , B. and open arrow in C.
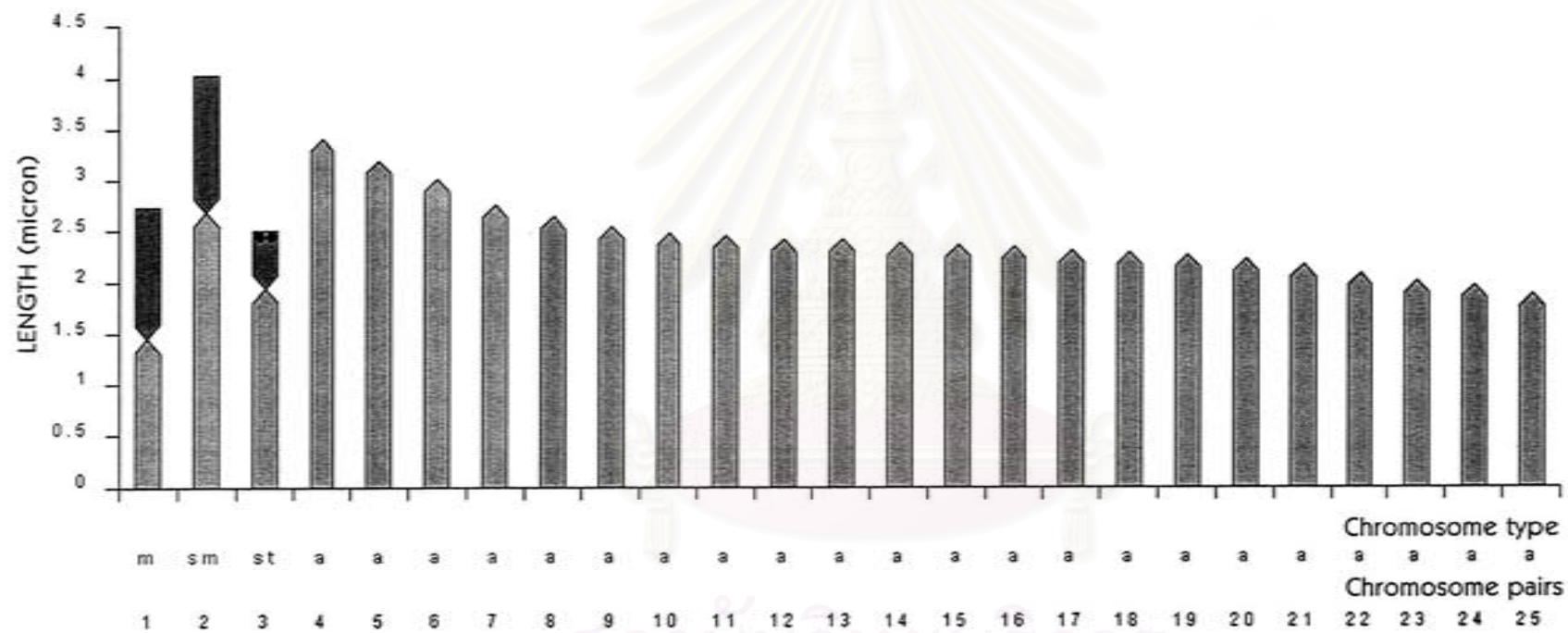
Figure 4.6 Idiogram of *Puntius brevis* based on the arm ratio and average chromosome length from five metaphase plates. ●●, indicated NORs
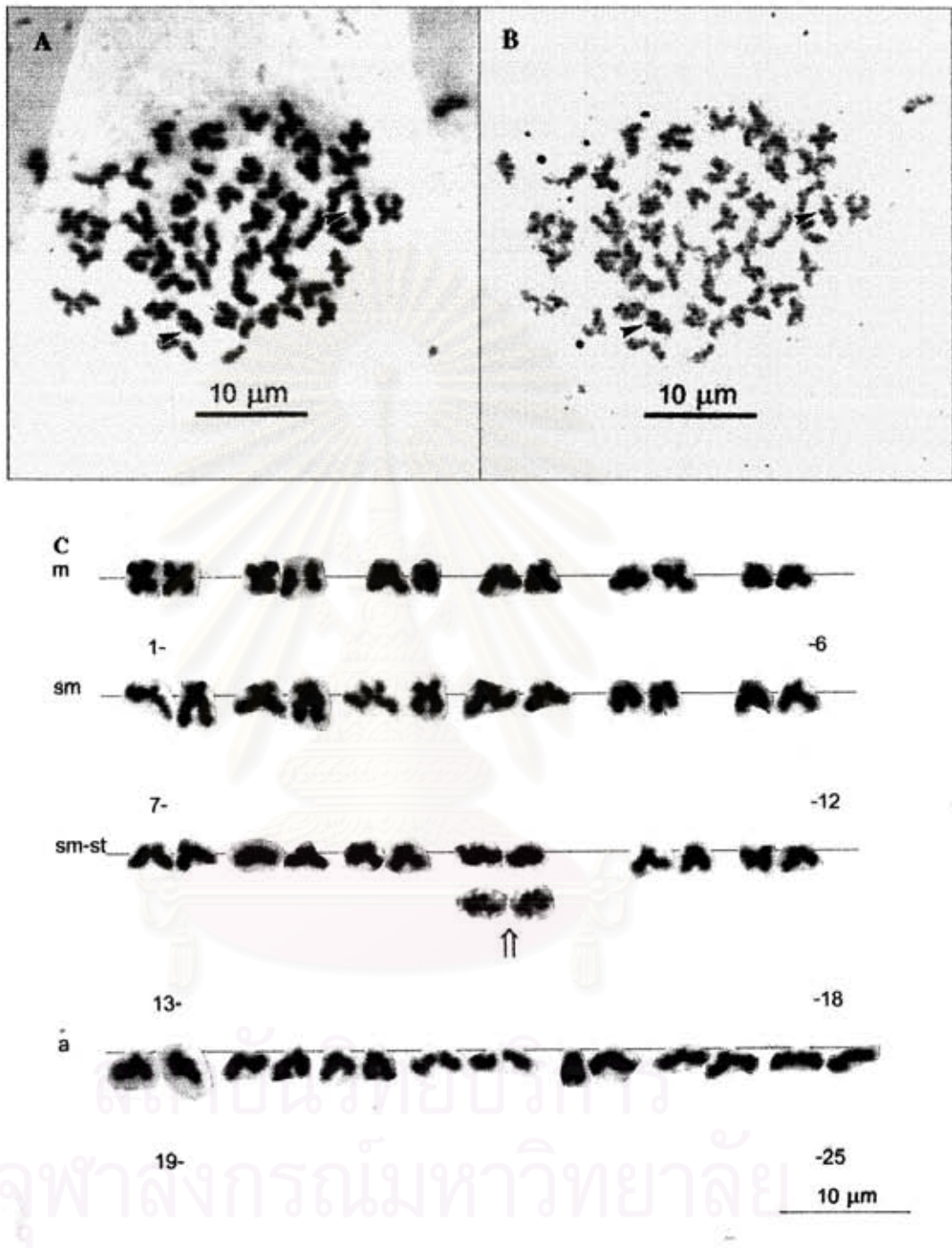
Figure 4.7 A. conventional Giemsa stained metaphase plate ,B. sequentially silver-stained metaphase plate and C. corresponding karyotype *of Systomus orphoides*; NOR bearing chromosomes indicated by arrow heads A., B. and open arrow in C.
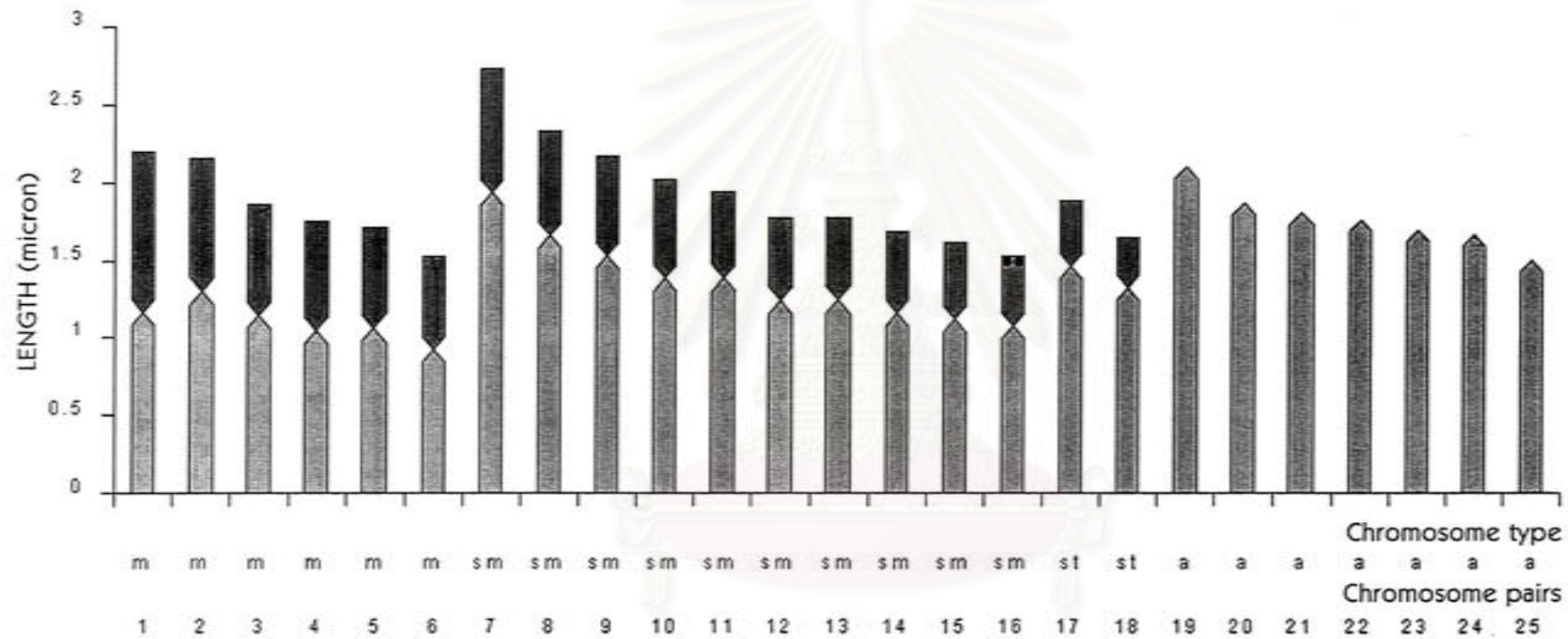
Figure 4.8 Idiogram of *Systomus orphoides* based on the arm ratio and average chromosome length from five metaphase plates. ●●, indicated NORs
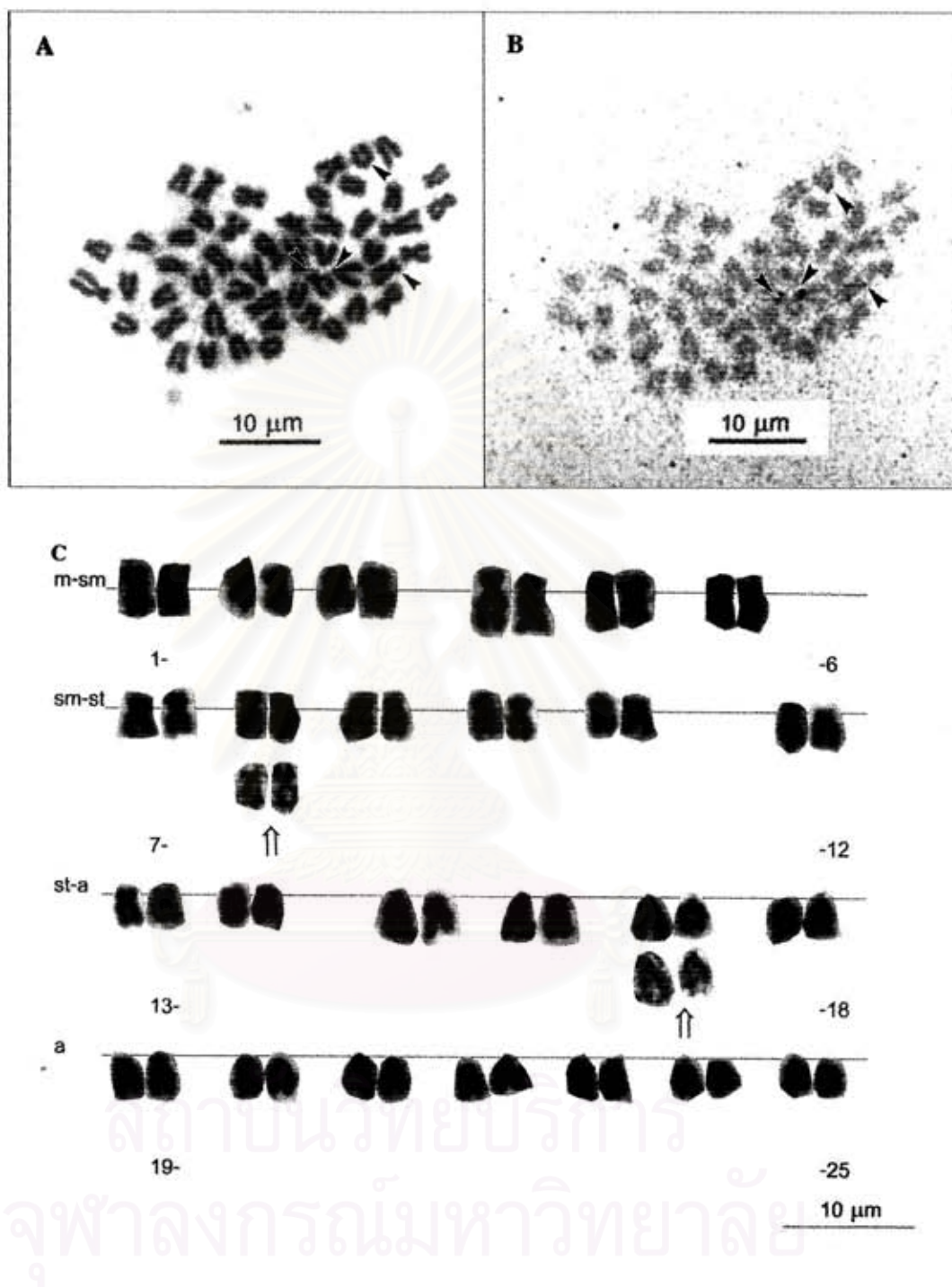
Figure 4.9 A. conventional Giemsa stained metaphase plate ,B. sequentially silver-stained metaphase plate and C. corresponding karyotype of Barbodes gonionotus; NOR bearing chromosomes indicated by arrow heads A. , B. and open arrow in C.
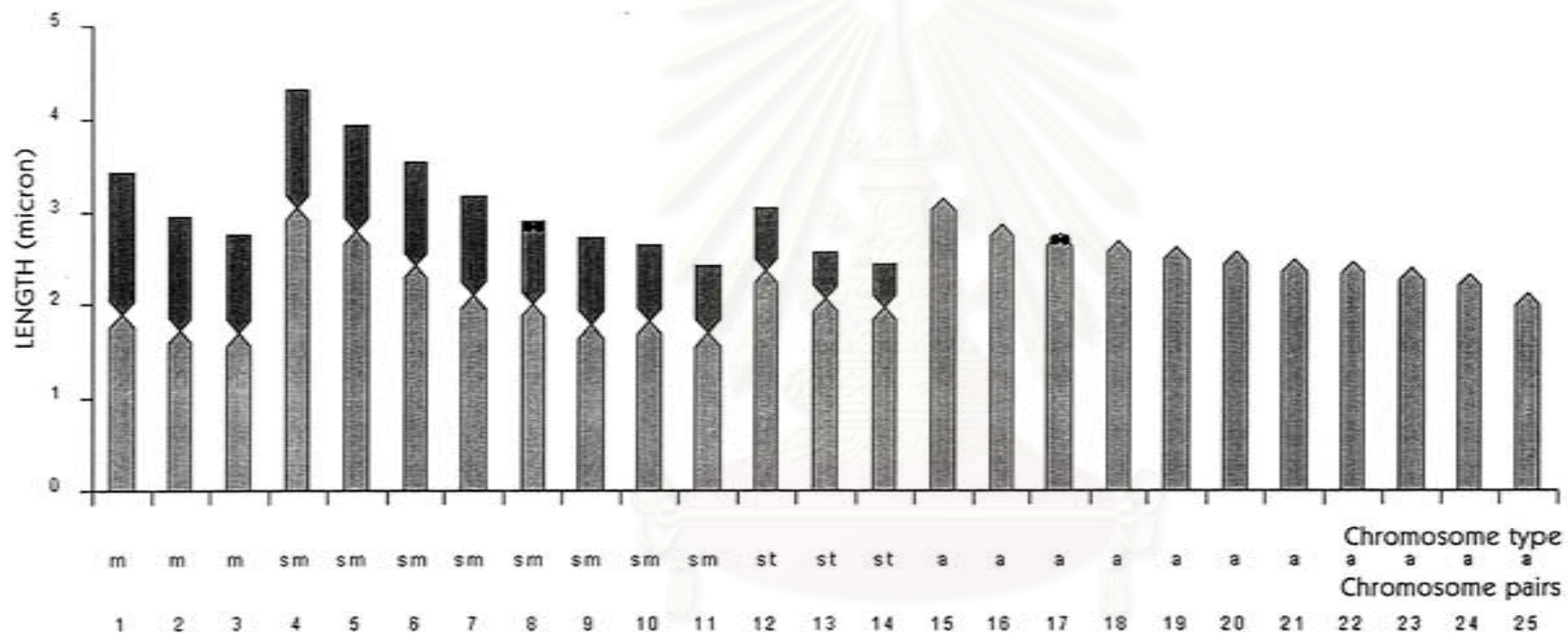
Figure 4.10 Idiogram of *Barbodes gonionotus* based on the arm ratio and average chromosome length from five metaphase plates. ●●, indicated NORs
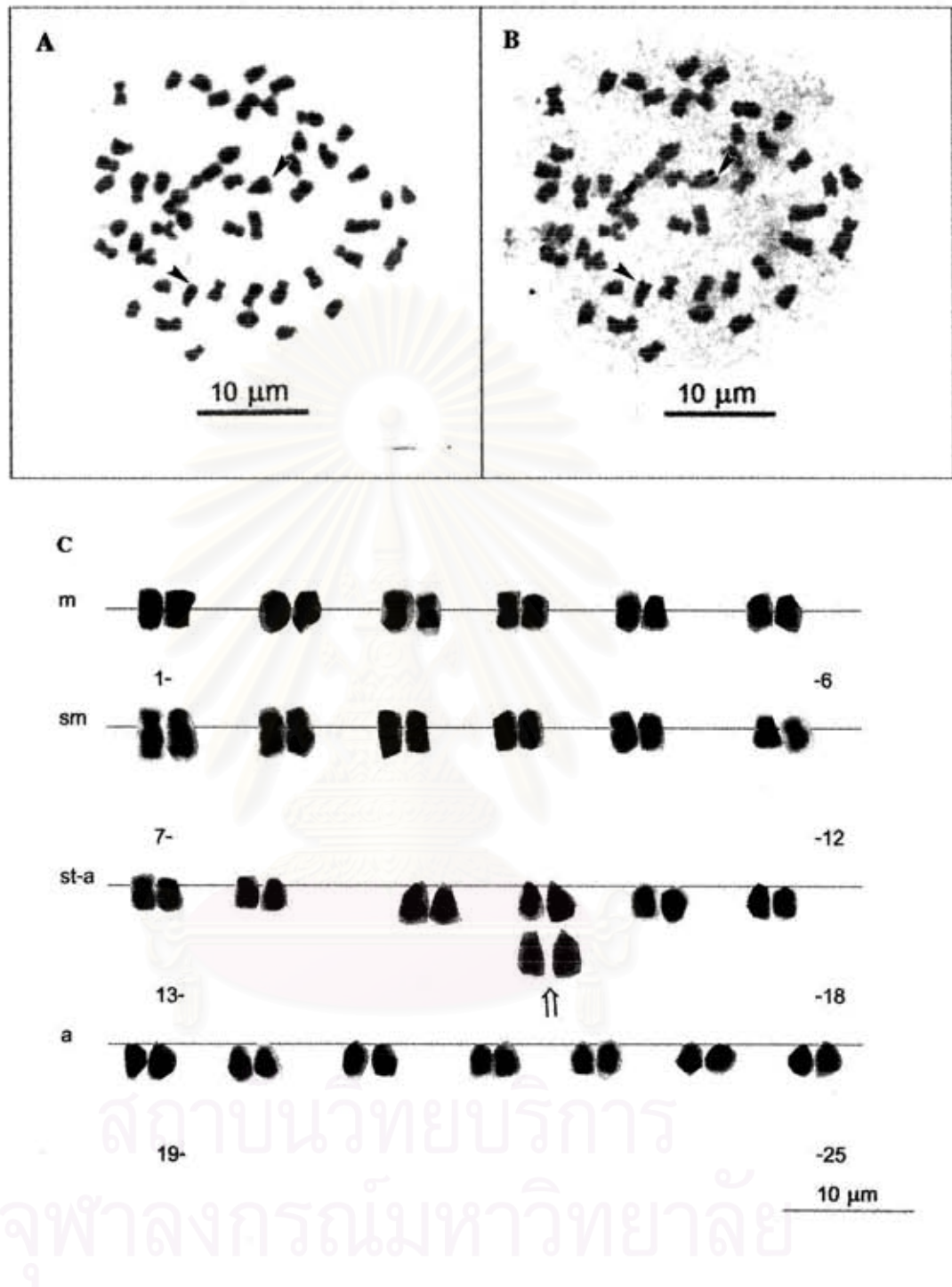
Figure 4.11 A. conventional Giemsa stained metaphase plate ,B. sequentially silver-stained metaphase plate and C. corresponding karyotype of Hypsibarbus wetmorei; NOR bearing chromosomes indicated by arrow heads A. , B. and open arrow in C.
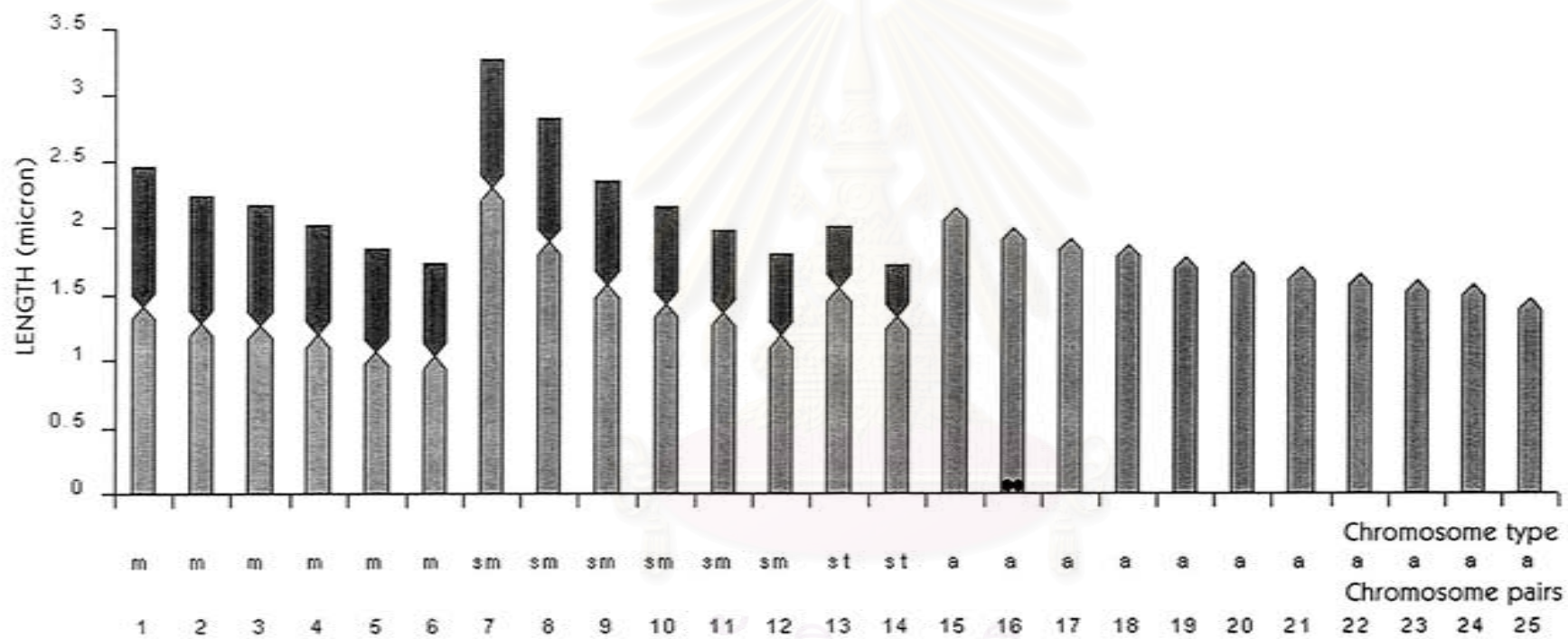
Figure 4.12 Idiogram of *Hypsibarbus wetmorei* based on the arm ratio and average chromosome length from five metaphase plates. ●●, indicated NORs

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

5.1.1 Karyotypes

In this study, all of four cyprinid species have similar diploid chromosome number, which is 50(2n=50), however they have different karyotypes as follows,

The karyotypes of *Puntius brevis* comprise of 2 metacentric chromosomes, 2 submetacentric chromosomes, 2 subtelocentric chromosomes and 44 acrocentric chromosomes (2m, 2sm, 2st and 44a), with their arm number(NF) as many as 54.

The karyotypes of *Systomus orphoides* comprise of 12m, 20sm, 4st and 14a with their NF as many as 82.

The karyotypes of *Barbodes gonionotus* comprise of 6m, 16sm, 6st and 22a with their NF as many as 72.

The karyotypes of *Hypsibarbus wetmorei* comprise of 12m, 12sm, 4st and 22a with their NF as many as 74.

5.1.2 NOR phenotypes

For distribution of nucleolus organizer regions (NORs) of four cyprinid species, the differences are as follows,

Puntius brevis, NORs have one pair which are located on the satellited regions of a subtelocentric pair on short arms.

Systomus orphoides, NORs have one pair which are located on the satellited regions of a submetacentric pair on short arms.

Barbodes gonionotus, NORs have two pairs which are located on the satellited regions of a submetacentric pair on short arms and on the satellited regions of an acrocentric pair on short arms.

Hypsibarbus wetmorei, NORs have one pair which are located on the satellited regions of an acrocentric pair on long arms.

5.2 Recommendations

1. Identification of four cyprinid species should be really careful since some close species have similar morphology also. It is essential that material specimens should be kept for checking of their scientific name.

2. Cell culture might be the better choice than direct method if it were found a suitable condition. At least, material specimens' sacrifices for cell culture will be less numbers than direct method.

3. Silver staining technique for NORs is sensitive to failure therefore a lot of metaphase plates should be prepared in this chromosome staining.

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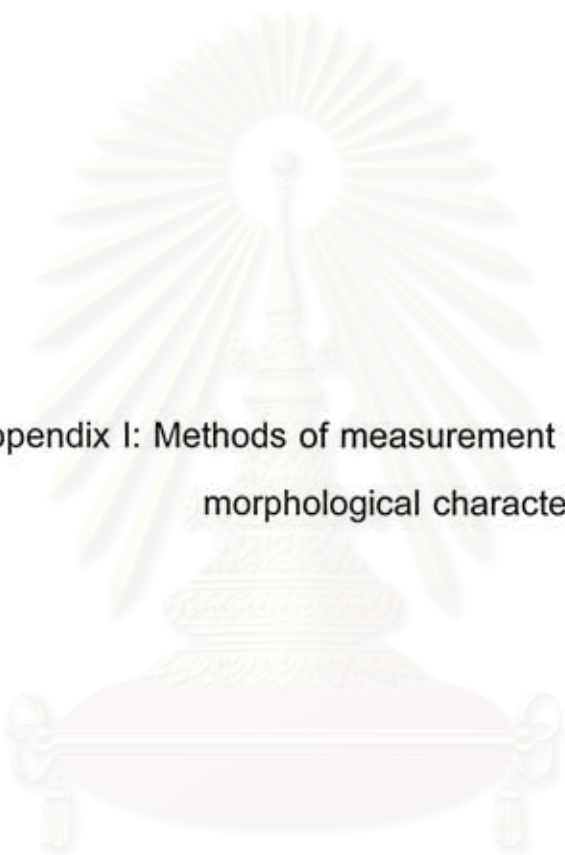
Appendices

Appendix I: Methods of measurement and counting fish

morphological characters

Methods of measurement and countings
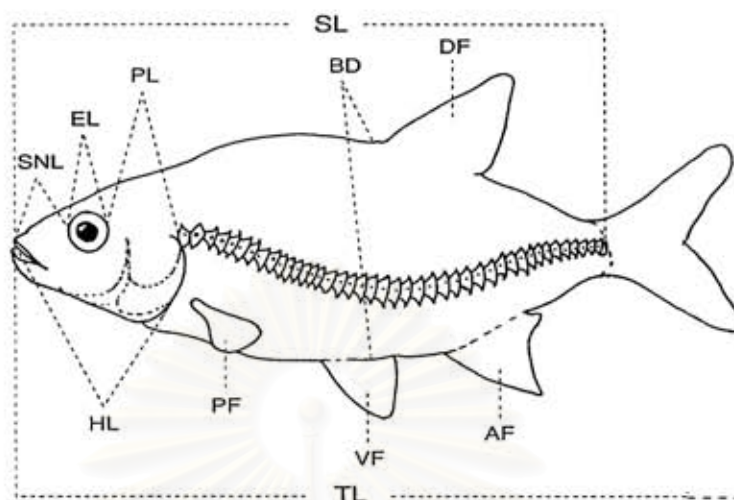
Figure I Topography of a cyprinid fish

TL= Total length: the straight distance from the tip of the snout to the tip of the caudal fin.

SL= Standard length: the straight distance from the snout to the junction of the posterior margin of the hypural bones or the base of the caudal fin or practically to the middle of the line of flexure at the caudal fin base.

BD= Body depth: the greatest depth usually taken vertically between the insertion of the first dorsal fin to the ventral profile.

HL= Head length: measured horizontally from the tip of the snout to the posterior edge of the operculum.

STL= Snout length: the straight distance from the tip of the snout to the anterior border of the eye.

EL= Eye diameter: the horizontal straight distance of diameter of eye.

PL= Post orbital length: the straight distance from the posterior edge of the operculum to the nearest point on the orbit.

DF= Dorsal fin rays
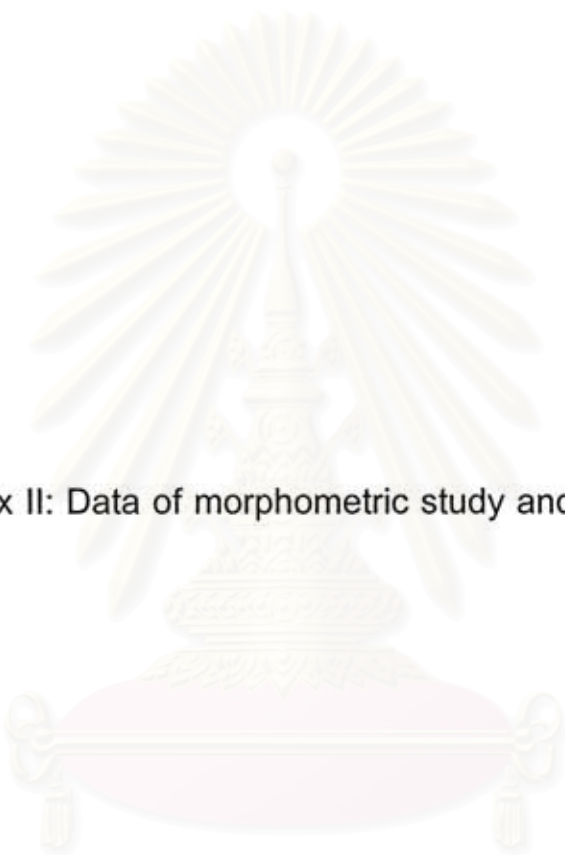
AF= Anal fin rays

VF= Pelvic fin rays

PF= Pectoral fin rays
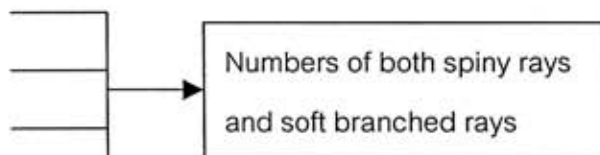
Numbers of both spiny rays and soft branched rays

Appendix II: Data of morphometric study and meristic count

Morphometric data of *Puntius brevis*

mm\specimens	1	2	3	4	5	6	7	8	9	10	mean	sd
total length(TL)	68	72	74	63	81	56	77	66	78	74	70.9	7.651434
standard length(SL)	59	63	65	56	67	49	63	59	69	65	61.5	5.911383
body depth(BD)	24	30	28	22	29	21	24	28	27	27	26	3.05505
head length(HL)	17.5	18	18	16	18	13	17	17	19	17	17.05	1.64063
snout length(STL)	3.45	4.55	3.9	3.8	3.5	2.7	2.15	3	3.15	3.6	3.38	0.671317
eye diameter(EL)	5.5	5.55	5.25	5.05	6.8	3.9	5.2	4.9	6	5.25	5.34	0.747143
postorbital length(PL)	7.6	7.6	7.6	6.05	7.2	5.25	7.1	7.35	8.55	7.35	7.165	0.911059
%TL/SL	115.2542	114.2857	113.8462	112.5	120.8955	114.2857	122.2222	111.8644	113.0435	113.8462	115.2044	3.498535
%SL/SL	100	100	100	100	100	100	100	100	100	100	100	0
%BD/SL	40.67797	47.61905	43.07692	39.28571	43.28358	42.85714	38.09524	47.45763	39.13043	41.53846	42.30221	3.279529
%HL/SL	29.66102	28.57143	27.69231	28.57143	26.86567	26.53061	26.98413	28.81356	27.53623	26.15385	27.73802	1.134482
%STL/SL	5.847458	7.222222	6	6.785714	5.223881	5.510204	3.412698	5.084746	4.565217	5.538462	5.51906	1.078431
%EL/SL	9.322034	8.809524	8.076923	9.017857	10.14925	7.959184	8.253968	8.305085	8.695652	8.076923	8.66664	0.688746
%PL/SL	12.88136	12.06349	11.69231	10.80357	10.74627	10.71429	11.26984	12.45763	12.3913	11.30769	11.63277	0.785865

Morphometric data of *Systomus orphoides*

mm\specimens	1	2	3	4	5	6	7	8	9	10	mean	sd
total length(TL)	98	88	108	98	99	87	89	104	119	112	100.2	10.6854
standard length(SL)	84	81	99	89	90	77	81	92	97	99	88.9	7.964505
body depth(BD)	33	33	37	35	32	27	32	34	34	36	33.3	2.750757
head length(HL)	23	22	26	22	23	21	21	23	24	25	23	1.632993
snout length(STL)	4.15	4.5	6.45	5.1	5.25	3.9	4.3	4.9	4.15	5.9	4.86	0.829592
eye diameter(EL)	5.5	6.1	6.55	6.95	6.65	6.4	5.5	6.1	5.75	6.85	6.235	0.531272
postorbital length(PL)	11	11	13.2	11.05	11.4	10.5	18.5	9.9	19	12.55	12.81	3.272002
%TL/SL	116.6667	108.642	109.0909	110.1124	110	112.987	109.8765	113.0435	122.6804	113.1313	112.6231	4.309178
%SL/SL	100	100	100	100	100	100	100	100	100	100	100	0
%BD/SL	39.28571	40.74074	37.37374	39.32584	35.55556	35.06494	39.50617	36.95652	35.05155	36.36364	37.52244	2.064314
%HL/SL	27.38095	27.16049	26.26263	24.7191	25.55556	27.27273	25.92593	25	24.74227	25.25253	25.92722	1.047427
%STL/SL	4.940476	5.555556	6.515152	5.730337	5.833333	5.064935	5.308642	5.326087	4.278351	5.959596	5.451246	0.61886
%EL/SL	6.547619	7.530864	6.616162	7.808989	7.388889	8.311688	6.790123	6.630435	5.927835	6.919192	7.04718	0.705097
%PL/SL	13.09524	13.58025	13.33333	12.41573	12.66667	13.63636	22.83951	10.76087	19.58763	12.67677	14.45924	3.732017

mm\specimens	1	2	3	4	5	6	7	8	9	10	mean	sd
total length(TL)	126	128	131	128	129	116	137	131	133	103	126.2	9.807027
standard length(SL)	114	102	114	114	113	101	119	112	118	88	109.5	9.617692
body depth(BD)	49	46	41	49	43	42	48	49	42	32	44.1	5.3427
head length(HL)	30.5	27	30	28	29	26	31	29	31	26	28.75	1.932902
snout length(STL)	5.75	2.85	5.1	4.2	5.75	5.25	5.5	4.7	7.45	4.3	5.085	1.211989
eye diameter(EL)	11.5	8.5	9.7	8.4	9.3	8.8	9.3	10.5	10	8.2	9.42	1.037947
postorbital length(PL)	12.65	12.05	13	13.45	13.25	12.65	13	13.4	12.5	13.5	12.945	0.476358
%TL/SL	110.5263	125.4902	114.9123	112.2807	114.1593	114.8515	115.1261	116.9643	112.7119	117.0455	115.4068	4.080144
%SL/SL	100	100	100	100	100	100	100	100	100	100	100	0
%BD/SL	42.98246	45.09804	35.96491	42.98246	38.0531	41.58416	40.33613	43.75	35.59322	36.36364	40.27081	3.5347
%HL/SL	26.75439	26.47059	26.31579	24.5614	25.66372	25.74257	26.05042	25.89286	26.27119	29.54545	26.32684	1.279269
%STL/SL	5.04386	2.794118	4.473684	3.684211	5.088496	5.19802	4.621849	4.196429	6.313559	4.886364	4.630059	0.947611
%EL/SL	10.08772	8.333333	8.508772	7.368421	8.230088	8.712871	7.815126	9.375	8.474576	9.318182	8.622409	0.795979
%PL/SL	11.09649	11.81373	11.40351	11.79825	11.72566	12.52475	10.92437	11.96429	10.59322	15.34091	11.91852	1.326261

Morphometric data of *Barbodes gonionotus*

Morphometric data of *Hypsibarbus wetmorei*

mm\specimens	1	2	3	4	5	6	7	8	9	10	mean	sd
total length(TL)	158	125	134	156	136	142	129	147	114	113	135.4	15.72118
standard length(SL)	140	109	113	134	118	123	108	122	98	94	115.9	14.60175
body depth(BD)	54	48	47	58	52	50	44	51	43	37	48.4	6.022181
head length(HL)	31	24	26	32	28	29	27	31	23	22	27.3	3.529243
snout length(STL)	7.8	4.25	6.35	7.3	5.6	7.95	5.8	6.95	4.75	5.85	6.26	1.244053
eye diameter(EL)	11.5	8.85	8.55	11.7	9.5	8.8	9.65	9.3	7.85	8	9.37	1.313223
postorbital length(PL)	13.5	10.75	11.5	12.95	10.7	13.5	11.55	13.05	12.5	9.6	11.96	1.341806
%TL/SL	112.8571	114.6789	118.5841	116.4179	115.2542	115.4472	119.4444	120.4918	116.3265	120.2128	116.9715	2.577535
%SL/SL	100	100	100	100	100	100	100	100	100	100	100	0
%BD/SL	38.57143	44.0367	41.59292	43.28358	44.0678	40.65041	40.74074	41.80328	43.87755	39.3617	41.79861	1.986577
%HL/SL	22.14286	22.01835	23.00885	23.8806	23.72881	23.57724	25	25.40984	23.46939	23.40426	23.56402	1.072283
%STL/SL	5.571429	3.899083	5.619469	5.447761	4.745763	6.463415	5.37037	5.696721	4.846939	6.223404	5.388435	0.742587
%EL/SL	8.214286	8.119266	7.566372	8.731343	8.050847	7.154472	8.935185	7.622951	8.010204	8.510638	8.091556	0.547145
%PL/SL	9.642857	9.862385	10.17699	9.664179	9.067797	10.97561	10.69444	10.69672	12.7551	10.21277	10.37489	1.017052

Meristic count data of *Puntius brevis*

numbers\specimens	1	2	3	4	5	6	7	8	9	10	mode
dorsal fin rays	9	9	9	9	9	9	9	9	9	9	9
anal fin rays	6	6	6	6	6	6	6	6	6	6	6
pelvic fin rays	9	9	9	9	9	9	9	9	9	9	9
pectoral fin rays	11	11	11	11	11	11	11	12	11	10	11

Meristic count data of *Systomus orphoides*

numbers\specimens	1	2	3	4	5	6	7	8	9	10	mode
dorsal fin rays	9	9	9	9	9	9	9	9	9	9	9
anal fin rays	6	6	6	6	6	6	6	6	6	6	6
pelvic fin rays	9	9	9	9	9	9	9	9	9	9	9
pectoral fin rays	13	13	13	13	13	13	12	13	13	13	13

Meristic count data of *Hypsibarbus wetmorei*

numbers\specimens	1	2	3	4	5	6	7	8	9	10	mode
dorsal fin rays	9	9	9	9	9	9	9	9	9	9	9
anal fin rays	6	6	6	6	6	6	6	6	6	6	6
pelvic fin rays	9	9	9	9	9	9	9	9	9	9	9
pectoral fin rays	12	12	12	12	12	12	12	12	12	12	12

Meristic count data of *Barbodes gonionotus*

numbers\specimens	1	2	3	4	5	6	7	8	9	10	mode
dorsal fin rays	9	9	9	9	9	9	9	9	9	9	9
anal fin rays	7	7	7	7	7	7	7	7	7	7	7
pelvic fin rays	9	9	9	9	9	9	9	8	9	9	9
pectoral fin rays	12	11	12	12	12	12	12	12	12	12	12

Appendix III: Data of karyotype and idiogram construction

		P1(cm)	P2(cm)	P3(cm)	P4(cm)	P5(cm)	long arm (l)	long arm (l)
pair	type	long arm (l)	long arm (l)	long arm (l)	long arm (l)	long arm (l)	sum of P	mean of P
1	m	0.525	1.145	0.475	0.62	0.55	3.315	0.663
1	m	0.53	1.05	0.48	0.55	0.57	3.18	0.636
2	sm	0.85	2	0.805	0.94	1.22	5.815	1.163
2	sm	1.025	2.315	0.875	0.89	1.2	6.305	1.261
3	st	0.625	1.58	0.695	0.785	0.63	4.315	0.863
3	st	0.86	1.515	0.68	0.66	0.625	4.34	0.868
4	a	1.42	2.575	1.08	1.165	1.46	7.7	1.54
4	a	1.365	2.565	1.05	1.125	1.44	7.545	1.509
5	a	1.33	2.52	1.025	1.095	1.39	7.36	1.472
5	a	1.235	2.51	0.945	1.065	1.265	7.02	1.404
6	a	1.22	2.49	0.94	1.06	1.255	6.965	1.393
6	a	1.165	2.29	0.925	1.05	1.135	6.565	1.313
7	a	1.1	2.095	0.92	1.035	1.115	6.265	1.253
7	a	1.085	2.025	0.915	1.025	1.075	6.125	1.225
8	a	1.08	2.02	0.89	1	1.025	6.015	1.203
8	a	1.07	1.88	0.89	0.975	1.005	5.82	1.164
9	a	1.055	1.865	0.88	0.975	1	5.775	1.155
9	a	1.035	1.845	0.865	0.955	0.995	5.695	1.139
10	a	1.025	1.8	0.85	0.955	0.99	5.62	1.124
10	a	0.995	1.79	0.845	0.94	0.98	5.55	1.11
11	a	0.99	1.785	0.845	0.93	0.975	5.525	1.105
11	a	0.985	1.76	0.84	0.925	0.965	5.475	1.095
12	a	0.985	1.755	0.84	0.92	0.955	5.455	1.091
12	a	0.985	1.75	0.83	0.91	0.94	5.415	1.083
13	a	0.985	1.75	0.82	0.905	0.94	5.4	1.08
13	a	0.98	1.745	0.81	0.905	0.94	5.38	1.076
14	a	0.98	1.735	0.81	0.905	0.935	5.365	1.073
14	a	0.97	1.735	0.805	0.9	0.93	5.34	1.068
15	a	0.95	1.73	0.8	0.9	0.93	5.31	1.062
15	a	0.95	1.73	0.78	0.9	0.93	5.29	1.058
16	a	0.945	1.715	0.78	0.895	0.925	5.26	1.052
16	a	0.945	1.71	0.78	0.895	0.925	5.255	1.051

Measurement data of chromosomes for *Puntius brevis*

		P1(cm)	P2(cm)	P3(cm)	P4(cm)	P5(cm)	long arm (l)	long arm (l)
pair	type	long arm (l)	long arm (l)	long arm (l)	long arm (l)	long arm (l)	sum of P	mean of P
17	a	0.93	1.68	0.77	0.89	0.925	5.195	1.039
17	a	0.925	1.68	0.765	0.885	0.915	5.17	1.034
18	a	0.92	1.67	0.765	0.88	0.905	5.14	1.028
18	a	0.915	1.66	0.76	0.87	0.905	5.11	1.022
19	a	0.915	1.66	0.755	0.865	0.905	5.1	1.02
19	a	0.905	1.66	0.74	0.865	0.885	5.055	1.011
20	a	0.88	1.655	0.74	0.855	0.88	5.01	1.002
20	a	0.88	1.64	0.735	0.84	0.88	4.975	0.995
21	a	0.85	1.61	0.73	0.835	0.835	4.86	0.972
21	a	0.84	1.6	0.73	0.83	0.815	4.815	0.963
22	a	0.83	1.575	0.71	0.785	0.795	4.695	0.939
22	a	0.82	1.535	0.705	0.775	0.79	4.625	0.925
23	a	0.81	1.5	0.7	0.73	0.785	4.525	0.905
23	a	0.8	1.49	0.7	0.725	0.77	4.485	0.897
24	a	0.79	1.49	0.7	0.72	0.76	4.46	0.892
24	a	0.76	1.455	0.7	0.715	0.735	4.365	0.873
25	a	0.745	1.455	0.7	0.71	0.725	4.335	0.867
25	a	0.685	1.44	0.635	0.67	0.7	4.13	0.826

Measurement data of chromosomes for *Puntius brevis*

Measurement data of chromosomes for *Puntius brevis*								
long arm (l)	P1(cm)	P2(cm)	P3(cm)	P4(cm)	P5(cm)	short arm (s)	short arm (s)	short arm (s)
SD of P	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of P	mean of P	SD of P
0.274468	0.49	1.05	0.405	0.5	0.51	2.955	0.591	0.259962
0.233838	0.515	1.02	0.345	0.445	0.45	2.775	0.555	0.26695
0.494894	0.455	0.88	0.455	0.45	0.505	2.745	0.549	0.186393
0.603525	0.58	1.24	0.4	0.465	0.48	3.165	0.633	0.345391
0.405996	0.205	0.48	0.195	0.22	0.19	1.29	0.258	0.124629
0.372938	0.225	0.49	0.18	0.205	0.185	1.285	0.257	0.131463
0.600885						0	#DIV/0!	#DIV/0!
0.612121						0	#DIV/0!	#DIV/0!
0.605626						0	#DIV/0!	#DIV/0!
0.631807						0	#DIV/0!	#DIV/0!
0.626195						0	#DIV/0!	#DIV/0!
0.554037						0	#DIV/0!	#DIV/0!
0.476925						0	#DIV/0!	#DIV/0!
0.452272						0	#DIV/0!	#DIV/0!
0.46192						0	#DIV/0!	#DIV/0!
0.405453						0	#DIV/0!	#DIV/0!
0.401917						0	#DIV/0!	#DIV/0!
0.399662						0	#DIV/0!	#DIV/0!
0.383526						0	#DIV/0!	#DIV/0!
0.384594						0	#DIV/0!	#DIV/0!
0.384301						0	#DIV/0!	#DIV/0!
0.375882						0	#DIV/0!	#DIV/0!
0.375123						0	#DIV/0!	#DIV/0!
0.377121						0	#DIV/0!	#DIV/0!
0.379391						0	#DIV/0!	#DIV/0!
0.379233						0	#DIV/0!	#DIV/0!
0.375277						0	#DIV/0!	#DIV/0!
0.3778						0	#DIV/0!	#DIV/0!
0.377849						0	#DIV/0!	#DIV/0!
0.381405						0	#DIV/0!	#DIV/0!
0.376092						0	#DIV/0!	#DIV/0!
0.373888						0	#DIV/0!	#DIV/0!
long arm (l)	P1(cm)	P2(cm)	P3(cm)	P4(cm)	P5(cm)	short arm (s)	short arm (s)	short arm (s)
SD of P	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of P	mean of P	SD of P

Measurement data of chromosomes for *Puntius brevis*								
long arm (l)	P1(cm)	P2(cm)	P3(cm)	P4(cm)	P5(cm)	short arm (s)	short arm (s)	short arm (s)
SD of P	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of P	mean of P	SD of P
0.364115						0	#DIV/0!	#DIV/0!
0.366715						0	#DIV/0!	#DIV/0!
0.364016						0	#DIV/0!	#DIV/0!
0.361915						0	#DIV/0!	#DIV/0!
0.363352						0	#DIV/0!	#DIV/0!
0.368466						0	#DIV/0!	#DIV/0!
0.369605						0	#DIV/0!	#DIV/0!
0.365411						0	#DIV/0!	#DIV/0!
0.359872						0	#DIV/0!	#DIV/0!
0.358741						0	#DIV/0!	#DIV/0!
0.358214						0	#DIV/0!	#DIV/0!
0.343602						0	#DIV/0!	#DIV/0!
0.335447						0	#DIV/0!	#DIV/0!
0.333759						0	#DIV/0!	#DIV/0!
0.33611						0	#DIV/0!	#DIV/0!
0.326125						0	#DIV/0!	#DIV/0!
0.329139						0	#DIV/0!	#DIV/0!
0.344082						0	#DIV/0!	#DIV/0!

Measurement data of chromosomes for *Puntius brevis*								
l+s	l/s	long arm	short arm	l+s(cm)	l/s	adjust	adjust	adjust
mean of P	mean of P	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)
1.254	1.121827	0.6495	0.573	1.2225	1.133887	1.443333	1.273333	2.716667
1.191	1.145946	0.6495	0.573	1.2225	1.133887	1.443333	1.273333	2.716667
1.712	2.118397	1.212	0.591	1.803	2.055249	2.693333	1.313333	4.006667
1.894	1.992101	1.212	0.591	1.803	2.055249	2.693333	1.313333	4.006667
1.121	3.344961	0.8655	0.2575	1.123	3.361197	1.923333	0.572222	2.495556
1.125	3.377432	0.8655	0.2575	1.123	3.361197	1.923333	0.572222	2.495556
#DIV/0!	#DIV/0!	1.5245	#DIV/0!	#DIV/0!	#DIV/0!	3.387778	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.5245	#DIV/0!	#DIV/0!	#DIV/0!	3.387778	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.438	#DIV/0!	#DIV/0!	#DIV/0!	3.195556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.438	#DIV/0!	#DIV/0!	#DIV/0!	3.195556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.353	#DIV/0!	#DIV/0!	#DIV/0!	3.006667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.353	#DIV/0!	#DIV/0!	#DIV/0!	3.006667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.239	#DIV/0!	#DIV/0!	#DIV/0!	2.753333	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.239	#DIV/0!	#DIV/0!	#DIV/0!	2.753333	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.1835	#DIV/0!	#DIV/0!	#DIV/0!	2.63	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.1835	#DIV/0!	#DIV/0!	#DIV/0!	2.63	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.147	#DIV/0!	#DIV/0!	#DIV/0!	2.548889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.147	#DIV/0!	#DIV/0!	#DIV/0!	2.548889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.117	#DIV/0!	#DIV/0!	#DIV/0!	2.482222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.117	#DIV/0!	#DIV/0!	#DIV/0!	2.482222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.1	#DIV/0!	#DIV/0!	#DIV/0!	2.444444	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.1	#DIV/0!	#DIV/0!	#DIV/0!	2.444444	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.087	#DIV/0!	#DIV/0!	#DIV/0!	2.415556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.087	#DIV/0!	#DIV/0!	#DIV/0!	2.415556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.078	#DIV/0!	#DIV/0!	#DIV/0!	2.395556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.078	#DIV/0!	#DIV/0!	#DIV/0!	2.395556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0705	#DIV/0!	#DIV/0!	#DIV/0!	2.378889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0705	#DIV/0!	#DIV/0!	#DIV/0!	2.378889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.06	#DIV/0!	#DIV/0!	#DIV/0!	2.355556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.06	#DIV/0!	#DIV/0!	#DIV/0!	2.355556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0515	#DIV/0!	#DIV/0!	#DIV/0!	2.336667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0515	#DIV/0!	#DIV/0!	#DIV/0!	2.336667	#DIV/0!	#DIV/0!
l+s	l/s	long arm	short arm	l+s(cm)	l/s	adjust	adjust	adjust
mean of P	mean of P	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)

Measurement data of chromosomes for *Puntius brevis*								
l+s	l/s	long arm	short arm	l+s(cm)	l/s	adjust	adjust	adjust
mean of P	mean of P	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)
#DIV/0!	#DIV/0!	1.0365	#DIV/0!	#DIV/0!	#DIV/0!	2.303333	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0365	#DIV/0!	#DIV/0!	#DIV/0!	2.303333	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.025	#DIV/0!	#DIV/0!	#DIV/0!	2.277778	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.025	#DIV/0!	#DIV/0!	#DIV/0!	2.277778	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0155	#DIV/0!	#DIV/0!	#DIV/0!	2.256667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0155	#DIV/0!	#DIV/0!	#DIV/0!	2.256667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.9985	#DIV/0!	#DIV/0!	#DIV/0!	2.218889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.9985	#DIV/0!	#DIV/0!	#DIV/0!	2.218889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.9675	#DIV/0!	#DIV/0!	#DIV/0!	2.15	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.9675	#DIV/0!	#DIV/0!	#DIV/0!	2.15	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.932	#DIV/0!	#DIV/0!	#DIV/0!	2.071111	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.932	#DIV/0!	#DIV/0!	#DIV/0!	2.071111	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.901	#DIV/0!	#DIV/0!	#DIV/0!	2.002222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.901	#DIV/0!	#DIV/0!	#DIV/0!	2.002222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.8825	#DIV/0!	#DIV/0!	#DIV/0!	1.961111	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.8825	#DIV/0!	#DIV/0!	#DIV/0!	1.961111	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.8465	#DIV/0!	#DIV/0!	#DIV/0!	1.881111	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.8465	#DIV/0!	#DIV/0!	#DIV/0!	1.881111	#DIV/0!	#DIV/0!

pair	type	S1(cm) long arm (l)	S2(cm) long arm (l)	S3(cm) long arm (l)	S4(cm) long arm (l)	S5(cm) long arm (l)	long arm (l) sum of S	long arm (l) mean of S
1	m	0.545	0.465	0.585	0.69	0.465	2.75	0.55
1	m	0.525	0.405	0.625	0.575	0.365	2.495	0.499
2	m	0.56	0.435	0.7	0.835	0.525	3.055	0.611
2	m	0.6	0.44	0.55	0.68	0.49	2.76	0.552
3	m	0.545	0.455	0.55	0.605	0.44	2.595	0.519
3	m	0.52	0.44	0.455	0.61	0.48	2.505	0.501
4	m	0.405	0.44	0.46	0.535	0.435	2.275	0.455
4	m	0.395	0.415	0.545	0.64	0.42	2.415	0.483
5	m	0.45	0.34	0.53	0.615	0.41	2.345	0.469
5	m	0.475	0.345	0.505	0.625	0.455	2.405	0.481
6	m	0.375	0.33	0.455	0.51	0.38	2.05	0.41
6	m	0.355	0.35	0.5	0.57	0.33	2.105	0.421
7	sm	0.82	0.8	1	1.15	0.75	4.52	0.904
7	sm	0.77	0.69	0.965	1.045	0.72	4.19	0.838
8	sm	0.79	0.7	0.795	0.705	0.715	3.705	0.741
8	sm	0.735	0.725	0.715	0.79	0.74	3.705	0.741
9	sm	0.655	0.645	0.715	0.765	0.54	3.32	0.664
9	sm	0.66	0.615	0.86	0.805	0.555	3.495	0.699
10	sm	0.65	0.57	0.715	0.765	0.475	3.175	0.635
10	sm	0.64	0.595	0.62	0.68	0.475	3.01	0.602
11	sm	0.65	0.56	0.76	0.68	0.53	3.18	0.636
11	sm	0.66	0.505	0.66	0.64	0.535	3	0.6
12	sm	0.6	0.475	0.59	0.635	0.47	2.77	0.554
12	sm	0.62	0.465	0.58	0.605	0.5	2.77	0.554
13	sm	0.53	0.49	0.555	0.575	0.59	2.74	0.548
13	sm	0.56	0.545	0.57	0.6	0.49	2.765	0.553
14	sm	0.55	0.455	0.55	0.615	0.44	2.61	0.522
14	sm	0.49	0.435	0.575	0.625	0.44	2.565	0.513
15	sm	0.54	0.44	0.5	0.58	0.415	2.475	0.495
15	sm	0.545	0.49	0.51	0.585	0.38	2.51	0.502
16	sm	0.49	0.46	0.65	0.515	0.38	2.495	0.499
16	sm	0.45	0.44	0.55	0.51	0.4	2.35	0.47

Measurement data of chromosomes for *Systomus orphoides*

		S1(cm)	S2(cm)	S3(cm)	S4(cm)	S5(cm)	long arm (l)	long arm (l)
pair	type	long arm (l)	long arm (l)	long arm (l)	long arm (l)	long arm (l)	sum of S	mean of S
17	st	0.63	0.51	0.71	0.82	0.725	3.395	0.679
17	st	0.63	0.545	0.76	0.65	0.56	3.145	0.629
18	st	0.6	0.49	0.71	0.72	0.485	3.005	0.601
18	st	0.555	0.475	0.66	0.665	0.52	2.875	0.575
19	a	0.915	0.79	1.6	1.005	0.82	5.13	1.026
19	a	0.875	0.715	0.96	0.965	0.815	4.33	0.866
20	a	0.875	0.71	0.925	0.945	0.805	4.26	0.852
20	a	0.815	0.705	0.915	0.905	0.79	4.13	0.826
21	a	0.81	0.7	0.905	0.88	0.785	4.08	0.816
21	a	0.8	0.69	0.87	0.875	0.78	4.015	0.803
22	a	0.79	0.66	0.865	0.87	0.775	3.96	0.792
22	a	0.755	0.655	0.86	0.865	0.755	3.89	0.778
23	a	0.735	0.65	0.83	0.865	0.75	3.83	0.766
23	a	0.73	0.645	0.825	0.855	0.73	3.785	0.757
24	a	0.72	0.645	0.825	0.85	0.69	3.73	0.746
24	a	0.715	0.635	0.8	0.825	0.66	3.635	0.727
25	a	0.665	0.61	0.775	0.81	0.64	3.5	0.7
25	a	0.635	0.555	0.735	0.685	0.61	3.22	0.644

Measurement data of chromosomes for *Systomus orphoides*

Measurement data of chromosomes for *Systomus orphoides*								
long arm (l)	S1(cm)	S2(cm)	S3(cm)	S4(cm)	S5(cm)	short arm (s)	short arm (s)	short arm (s)
SD of S	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of S	mean of S	SD of S
0.093941	0.45	0.4	0.52	0.585	0.415	2.37	0.474	0.077411
0.110815	0.465	0.39	0.525	0.525	0.35	2.255	0.451	0.079168
0.157377	0.38	0.33	0.465	0.49	0.36	2.025	0.405	0.069101
0.093648	0.355	0.34	0.42	0.4	0.325	1.84	0.368	0.040404
0.069588	0.325	0.285	0.375	0.43	0.28	1.695	0.339	0.063581
0.068044	0.31	0.26	0.315	0.425	0.285	1.595	0.319	0.063186
0.048862	0.295	0.26	0.295	0.47	0.255	1.575	0.315	0.088671
0.105865	0.36	0.245	0.335	0.385	0.265	1.59	0.318	0.060581
0.106677	0.27	0.245	0.325	0.37	0.245	1.455	0.291	0.054932
0.100648	0.285	0.22	0.31	0.37	0.28	1.465	0.293	0.054268
0.071676	0.25	0.195	0.29	0.3	0.255	1.29	0.258	0.041322
0.107378	0.325	0.21	0.3	0.345	0.2	1.38	0.276	0.066839
0.166823	0.285	0.295	0.45	0.48	0.255	1.765	0.353	0.103839
0.157663	0.26	0.285	0.535	0.395	0.275	1.75	0.35	0.116404
0.047355	0.31	0.285	0.325	0.355	0.25	1.525	0.305	0.039843
0.029026	0.31	0.26	0.295	0.415	0.26	1.54	0.308	0.063699
0.084587	0.3	0.355	0.295	0.305	0.215	1.47	0.294	0.050299
0.128909	0.32	0.29	0.3	0.345	0.195	1.45	0.29	0.057118
0.115488	0.28	0.295	0.365	0.255	0.23	1.425	0.285	0.051113
0.077508	0.29	0.29	0.325	0.31	0.25	1.465	0.293	0.028196
0.092898	0.25	0.195	0.3	0.335	0.185	1.265	0.253	0.065058
0.074246	0.285	0.22	0.265	0.315	0.185	1.27	0.254	0.051769
0.076273	0.205	0.215	0.315	0.265	0.23	1.23	0.246	0.044777
0.067952	0.215	0.2	0.305	0.295	0.205	1.22	0.244	0.051527
0.039465	0.24	0.175	0.28	0.295	0.205	1.195	0.239	0.050175
0.040559	0.235	0.22	0.315	0.295	0.175	1.24	0.248	0.056965
0.073195	0.22	0.185	0.31	0.28	0.175	1.17	0.234	0.059097
0.084158	0.21	0.185	0.325	0.27	0.19	1.18	0.236	0.060146
0.068374	0.25	0.17	0.26	0.245	0.2	1.125	0.225	0.038406
0.077185	0.25	0.17	0.28	0.25	0.2	1.15	0.23	0.044159
0.098514	0.195	0.185	0.275	0.2	0.17	1.025	0.205	0.040774
0.059582	0.215	0.155	0.225	0.195	0.18	0.97	0.194	0.027928
long arm (l)	S1(cm)	S2(cm)	S3(cm)	S4(cm)	S5(cm)	short arm (s)	short arm (s)	short arm (s)
SD of S	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of S	mean of S	SD of S

long arm (l) SD of S	S1(cm) short arm (s)	S2(cm) short arm (s)	S3(cm) short arm (s)	S4(cm) short arm (s)	S5(cm) short arm (s)	short arm (s) sum of S	short arm (s) mean of S	short arm (s) SD of S
0.116103	0.19	0.135	0.23	0.255	0.145	0.955	0.191	0.05213
0.085761	0.2	0.155	0.245	0.21	0.115	0.925	0.185	0.050621
0.11382	0.125	0.15	0.22	0.17	0.11	0.775	0.155	0.043012
0.084779	0.12	0.125	0.215	0.13	0.14	0.73	0.146	0.039275
0.331802						0	#DIV/0!	#DIV/0!
0.105024						0	#DIV/0!	#DIV/0!
0.096021						0	#DIV/0!	#DIV/0!
0.08692						0	#DIV/0!	#DIV/0!
0.081348						0	#DIV/0!	#DIV/0!
0.075796						0	#DIV/0!	#DIV/0!
0.085338						0	#DIV/0!	#DIV/0!
0.087293						0	#DIV/0!	#DIV/0!
0.084513						0	#DIV/0!	#DIV/0!
0.084009						0	#DIV/0!	#DIV/0!
0.088133						0	#DIV/0!	#DIV/0!
0.083711						0	#DIV/0!	#DIV/0!
0.087536						0	#DIV/0!	#DIV/0!
0.069138						0	#DIV/0!	#DIV/0!

Measurement data of chromosomes for *Systomus orphoides*								
l+s	l/s	long arm	short arm	l+s	l/s	adjust	adjust	adjust
mean of S	mean of S	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)
1.024	1.160338	0.5245	0.4625	0.987	1.133384	1.165556	1.027778	2.193333
0.95	1.10643	0.5245	0.4625	0.987	1.133384	1.165556	1.027778	2.193333
1.016	1.508642	0.5815	0.3865	0.968	1.504321	1.292222	0.858889	2.151111
0.92	1.5	0.5815	0.3865	0.968	1.504321	1.292222	0.858889	2.151111
0.858	1.530973	0.51	0.329	0.839	1.550753	1.133333	0.731111	1.864444
0.82	1.570533	0.51	0.329	0.839	1.550753	1.133333	0.731111	1.864444
0.77	1.444444	0.469	0.3165	0.7855	1.481656	1.042222	0.703333	1.745556
0.801	1.518868	0.469	0.3165	0.7855	1.481656	1.042222	0.703333	1.745556
0.76	1.611684	0.475	0.292	0.767	1.626661	1.055556	0.648889	1.704444
0.774	1.641638	0.475	0.292	0.767	1.626661	1.055556	0.648889	1.704444
0.668	1.589147	0.4155	0.267	0.6825	1.557255	0.923333	0.593333	1.516667
0.697	1.525362	0.4155	0.267	0.6825	1.557255	0.923333	0.593333	1.516667
1.257	2.560907	0.871	0.3515	1.2225	2.477596	1.935556	0.781111	2.716667
1.188	2.394286	0.871	0.3515	1.2225	2.477596	1.935556	0.781111	2.716667
1.046	2.429508	0.741	0.3065	1.0475	2.417676	1.646667	0.681111	2.327778
1.049	2.405844	0.741	0.3065	1.0475	2.417676	1.646667	0.681111	2.327778
0.958	2.258503	0.6815	0.292	0.9735	2.334424	1.514444	0.648889	2.163333
0.989	2.410345	0.6815	0.292	0.9735	2.334424	1.514444	0.648889	2.163333
0.92	2.22807	0.6185	0.289	0.9075	2.141339	1.374444	0.642222	2.016667
0.895	2.054608	0.6185	0.289	0.9075	2.141339	1.374444	0.642222	2.016667
0.889	2.513834	0.618	0.2535	0.8715	2.438019	1.373333	0.563333	1.936667
0.854	2.362205	0.618	0.2535	0.8715	2.438019	1.373333	0.563333	1.936667
0.8	2.252033	0.554	0.245	0.799	2.261262	1.231111	0.544444	1.775556
0.798	2.270492	0.554	0.245	0.799	2.261262	1.231111	0.544444	1.775556
0.787	2.292887	0.5505	0.2435	0.794	2.261363	1.223333	0.541111	1.764444
0.801	2.229839	0.5505	0.2435	0.794	2.261363	1.223333	0.541111	1.764444
0.756	2.230769	0.5175	0.235	0.7525	2.202249	1.15	0.522222	1.672222
0.749	2.173729	0.5175	0.235	0.7525	2.202249	1.15	0.522222	1.672222
0.72	2.2	0.4985	0.2275	0.726	2.191304	1.107778	0.505556	1.613333
0.732	2.182609	0.4985	0.2275	0.726	2.191304	1.107778	0.505556	1.613333
0.704	2.434146	0.4845	0.1995	0.684	2.428413	1.076667	0.443333	1.52
0.664	2.42268	0.4845	0.1995	0.684	2.428413	1.076667	0.443333	1.52

Measurement data of chromosomes for *Systomus orphoides*								
l+s	l/s	long arm	short arm	l+s	l/s	adjust	adjust	adjust
mean of S	mean of S	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)
0.87	3.554974	0.654	0.188	0.842	3.477487	1.453333	0.417778	1.871111
0.814	3.4	0.654	0.188	0.842	3.477487	1.453333	0.417778	1.871111
0.756	3.877419	0.588	0.1505	0.7385	3.907888	1.306667	0.334444	1.641111
0.721	3.938356	0.588	0.1505	0.7385	3.907888	1.306667	0.334444	1.641111
#DIV/0!	#DIV/0!	0.946	#DIV/0!	#DIV/0!	#DIV/0!	2.102222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.946	#DIV/0!	#DIV/0!	#DIV/0!	2.102222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.839	#DIV/0!	#DIV/0!	#DIV/0!	1.864444	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.839	#DIV/0!	#DIV/0!	#DIV/0!	1.864444	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.8095	#DIV/0!	#DIV/0!	#DIV/0!	1.798889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.8095	#DIV/0!	#DIV/0!	#DIV/0!	1.798889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.785	#DIV/0!	#DIV/0!	#DIV/0!	1.744444	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.785	#DIV/0!	#DIV/0!	#DIV/0!	1.744444	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.7615	#DIV/0!	#DIV/0!	#DIV/0!	1.692222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.7615	#DIV/0!	#DIV/0!	#DIV/0!	1.692222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.7365	#DIV/0!	#DIV/0!	#DIV/0!	1.636667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.7365	#DIV/0!	#DIV/0!	#DIV/0!	1.636667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.672	#DIV/0!	#DIV/0!	#DIV/0!	1.493333	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.672	#DIV/0!	#DIV/0!	#DIV/0!	1.493333	#DIV/0!	#DIV/0!

pair	type	H1 long arm (l)	H2 long arm (l)	H3 long arm (l)	H4 long arm (l)	H5 long arm (l)	long arm (l) sum of H	long arm (l) mean of H
1	m	0.7	0.595	0.675	0.635	0.72	3.325	0.665
1	m	0.565	0.545	0.595	0.585	0.7	2.99	0.598
2	m	0.575	0.49	0.68	0.54	0.58	2.865	0.573
2	m	0.565	0.52	0.68	0.49	0.63	2.885	0.577
3	m	0.65	0.415	0.7	0.5	0.675	2.94	0.588
3	m	0.54	0.4	0.68	0.49	0.655	2.765	0.553
4	m	0.6	0.4	0.655	0.47	0.66	2.785	0.557
4	m	0.605	0.4	0.53	0.465	0.61	2.61	0.522
5	m	0.57	0.4	0.525	0.41	0.5	2.405	0.481
5	m	0.55	0.4	0.5	0.455	0.48	2.385	0.477
6	m	0.5	0.415	0.49	0.43	0.445	2.28	0.456
6	m	0.55	0.38	0.48	0.43	0.535	2.375	0.475
7	sm	1.1	0.85	1.135	0.99	1.21	5.285	1.057
7	sm	1.025	0.87	1.065	0.785	1.285	5.03	1.006
8	sm	0.96	0.825	0.95	0.705	0.99	4.43	0.886
8	sm	0.9	0.63	0.935	0.74	0.925	4.13	0.826
9	sm	0.81	0.7	0.675	0.695	0.8	3.68	0.736
9	sm	0.745	0.61	0.65	0.535	0.8	3.34	0.668
10	sm	0.7	0.61	0.72	0.685	0.76	3.475	0.695
10	sm	0.675	0.475	0.605	0.51	0.665	2.93	0.586
11	sm	0.69	0.6	0.685	0.565	0.64	3.18	0.636
11	sm	0.68	0.5	0.61	0.56	0.6	2.95	0.59
12	sm	0.56	0.475	0.55	0.4	0.6	2.585	0.517
12	sm	0.735	0.455	0.58	0.455	0.55	2.775	0.555
13	st	0.76	0.58	0.66	0.68	0.795	3.475	0.695
13	st	0.68	0.56	0.785	0.68	0.785	3.49	0.698
14	st	0.72	0.48	0.59	0.635	0.68	3.105	0.621
14	st	0.6	0.5	0.56	0.645	0.58	2.885	0.577
15	a	1.145	0.8	0.98	0.915	1.175	5.015	1.003
15	a	1.11	0.765	0.98	0.89	0.915	4.66	0.932
16	a	1.03	0.735	0.95	0.875	0.9	4.49	0.898
16	a	1.025	0.685	0.95	0.855	0.885	4.4	0.88

Measurement data of chromosomes for *Hypsibarbus wetmorei*

Measurement data of chromosomes for *Hypsibarbus wetmorei*							
		H1	H2	H3	H4	H5	long arm (l)	long arm (l)
pair	type	long arm (l)	long arm (l)	long arm (l)	long arm (l)	long arm (l)	sum of H	mean of H
17	a	1.02	0.675	0.95	0.8	0.855	4.3	0.86
17	a	1.01	0.665	0.93	0.79	0.855	4.25	0.85
18	a	1.01	0.655	0.93	0.775	0.845	4.215	0.843
18	a	1	0.65	0.9	0.725	0.83	4.105	0.821
19	a	0.99	0.65	0.9	0.705	0.82	4.065	0.813
19	a	0.925	0.65	0.885	0.68	0.8	3.94	0.788
20	a	0.915	0.65	0.88	0.67	0.8	3.915	0.783
20	a	0.91	0.65	0.865	0.655	0.795	3.875	0.775
21	a	0.88	0.645	0.865	0.65	0.785	3.825	0.765
21	a	0.875	0.635	0.805	0.645	0.76	3.72	0.744
22	a	0.875	0.635	0.8	0.64	0.755	3.705	0.741
22	a	0.87	0.62	0.8	0.61	0.725	3.625	0.725
23	a	0.86	0.62	0.795	0.61	0.68	3.565	0.713
23	a	0.855	0.615	0.795	0.61	0.68	3.555	0.711
24	a	0.815	0.6	0.785	0.61	0.67	3.48	0.696
24	a	0.815	0.595	0.77	0.6	0.655	3.435	0.687
25	a	0.8	0.55	0.685	0.595	0.65	3.28	0.656
25	a	0.795	0.55	0.675	0.57	0.635	3.225	0.645

Measurement data of chromosomes for *Hypsibarbus wetmorei*								
long arm (l)	H1	H2	H3	H4	H5	short arm (s)	short arm (s)	short arm (s)
SD of H	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of H	mean of H	SD of H
0.050374	0.545	0.425	0.505	0.4	0.525	2.4	0.48	0.063836
0.060166	0.51	0.4	0.52	0.35	0.58	2.36	0.472	0.094181
0.069785	0.475	0.4	0.44	0.37	0.555	2.24	0.448	0.071816
0.07807	0.44	0.335	0.44	0.355	0.5	2.07	0.414	0.067952
0.124127	0.435	0.33	0.455	0.39	0.44	2.05	0.41	0.050867
0.116276	0.395	0.36	0.455	0.38	0.43	2.02	0.404	0.038308
0.11649	0.375	0.315	0.43	0.35	0.415	1.885	0.377	0.046984
0.090595	0.36	0.3	0.395	0.35	0.38	1.785	0.357	0.036332
0.073858	0.345	0.275	0.4	0.3	0.415	1.735	0.347	0.060889
0.055408	0.34	0.25	0.39	0.305	0.45	1.735	0.347	0.076942
0.037316	0.36	0.245	0.365	0.275	0.315	1.56	0.312	0.052393
0.071239	0.345	0.23	0.405	0.255	0.32	1.555	0.311	0.070303
0.14025	0.45	0.4	0.52	0.4	0.58	2.35	0.47	0.07874
0.193016	0.4	0.41	0.475	0.315	0.465	2.065	0.413	0.063894
0.119237	0.485	0.38	0.5	0.315	0.415	2.095	0.419	0.076273
0.135065	0.48	0.345	0.4	0.355	0.44	2.02	0.404	0.056943
0.063777	0.36	0.245	0.395	0.395	0.44	1.835	0.367	0.073875
0.105747	0.3	0.27	0.38	0.3	0.41	1.66	0.332	0.059749
0.055227	0.355	0.325	0.4	0.335	0.325	1.74	0.348	0.031544
0.090305	0.34	0.27	0.345	0.28	0.325	1.56	0.312	0.034749
0.054014	0.28	0.205	0.325	0.25	0.34	1.4	0.28	0.055114
0.066332	0.275	0.23	0.295	0.24	0.34	1.38	0.276	0.044357
0.079498	0.27	0.26	0.245	0.22	0.3	1.295	0.259	0.029665
0.115163	0.39	0.235	0.295	0.23	0.24	1.39	0.278	0.067879
0.085	0.19	0.145	0.2	0.205	0.26	1	0.2	0.041079
0.093314	0.165	0.14	0.26	0.22	0.26	1.045	0.209	0.054818
0.092628	0.2	0.13	0.19	0.175	0.22	0.915	0.183	0.033838
0.053339	0.185	0.125	0.18	0.165	0.155	0.81	0.162	0.023875
0.157504						0	#DIV/0!	#DIV/0!
0.126422						0	#DIV/0!	#DIV/0!
0.108662						0	#DIV/0!	#DIV/0!
0.127083						0	#DIV/0!	#DIV/0!

Measurement data of chromosomes for *Hypsibarbus wetmorei*								
long arm (l)	H1	H2	H3	H4	H5	short arm (s)	short arm (s)	short arm (s)
SD of H	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of H	mean of H	SD of H
0.133744						0	#DIV/0!	#DIV/0!
0.132146						0	#DIV/0!	#DIV/0!
0.137323						0	#DIV/0!	#DIV/0!
0.138582						0	#DIV/0!	#DIV/0!
0.138906						0	#DIV/0!	#DIV/0!
0.12148						0	#DIV/0!	#DIV/0!
0.119979						0	#DIV/0!	#DIV/0!
0.119111						0	#DIV/0!	#DIV/0!
0.113192						0	#DIV/0!	#DIV/0!
0.103465						0	#DIV/0!	#DIV/0!
0.103767						0	#DIV/0!	#DIV/0!
0.112805						0	#DIV/0!	#DIV/0!
0.110318						0	#DIV/0!	#DIV/0!
0.109738						0	#DIV/0!	#DIV/0!
0.099209						0	#DIV/0!	#DIV/0!
0.100412						0	#DIV/0!	#DIV/0!
0.095616						0	#DIV/0!	#DIV/0!
0.09766						0	#DIV/0!	#DIV/0!

Measurement data of chromosomes for *Hypsibarbus wetmorei*								
l+s	l/s	long arm	short arm	l+s	l/s	adjust	adjust	adjust
mean of H	mean of H	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)
1.145	1.385417	0.6315	0.476	1.1075	1.326183	1.403333	1.057778	2.461111
1.07	1.266949	0.6315	0.476	1.1075	1.326183	1.403333	1.057778	2.461111
1.021	1.279018	0.575	0.431	1.006	1.336369	1.277778	0.957778	2.235556
0.991	1.39372	0.575	0.431	1.006	1.336369	1.277778	0.957778	2.235556
0.998	1.434146	0.5705	0.407	0.9775	1.401479	1.267778	0.904444	2.172222
0.957	1.368812	0.5705	0.407	0.9775	1.401479	1.267778	0.904444	2.172222
0.934	1.477454	0.5395	0.367	0.9065	1.469819	1.198889	0.815556	2.014444
0.879	1.462185	0.5395	0.367	0.9065	1.469819	1.198889	0.815556	2.014444
0.828	1.386167	0.479	0.347	0.826	1.380403	1.064444	0.771111	1.835556
0.824	1.37464	0.479	0.347	0.826	1.380403	1.064444	0.771111	1.835556
0.768	1.461538	0.4655	0.3115	0.777	1.494435	1.034444	0.692222	1.726667
0.786	1.527331	0.4655	0.3115	0.777	1.494435	1.034444	0.692222	1.726667
1.527	2.248936	1.0315	0.4415	1.473	2.342386	2.292222	0.981111	3.273333
1.419	2.435835	1.0315	0.4415	1.473	2.342386	2.292222	0.981111	3.273333
1.305	2.114558	0.856	0.4115	1.2675	2.079556	1.902222	0.914444	2.816667
1.23	2.044554	0.856	0.4115	1.2675	2.079556	1.902222	0.914444	2.816667
1.103	2.00545	0.702	0.3495	1.0515	2.008749	1.56	0.776667	2.336667
1	2.012048	0.702	0.3495	1.0515	2.008749	1.56	0.776667	2.336667
1.043	1.997126	0.6405	0.33	0.9705	1.937666	1.423333	0.733333	2.156667
0.898	1.878205	0.6405	0.33	0.9705	1.937666	1.423333	0.733333	2.156667
0.916	2.271429	0.613	0.278	0.891	2.204555	1.362222	0.617778	1.98
0.866	2.137681	0.613	0.278	0.891	2.204555	1.362222	0.617778	1.98
0.776	1.996139	0.536	0.2685	0.8045	1.996271	1.191111	0.596667	1.787778
0.833	1.996403	0.536	0.2685	0.8045	1.996271	1.191111	0.596667	1.787778
0.895	3.475	0.6965	0.2045	0.901	3.407356	1.547778	0.454444	2.002222
0.907	3.339713	0.6965	0.2045	0.901	3.407356	1.547778	0.454444	2.002222
0.804	3.393443	0.599	0.1725	0.7715	3.477586	1.331111	0.383333	1.714444
0.739	3.561728	0.599	0.1725	0.7715	3.477586	1.331111	0.383333	1.714444
#DIV/0!	#DIV/0!	0.9675	#DIV/0!	#DIV/0!	#DIV/0!	2.15	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.9675	#DIV/0!	#DIV/0!	#DIV/0!	2.15	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.889	#DIV/0!	#DIV/0!	#DIV/0!	1.975556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.889	#DIV/0!	#DIV/0!	#DIV/0!	1.975556	#DIV/0!	#DIV/0!

Measurement data of chromosomes for *Hypsibarbus wetmorei*								
l+s	l/s	long arm	short arm	l+s	l/s	adjust	adjust	adjust
mean of H	mean of H	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)
#DIV/0!	#DIV/0!	0.855	#DIV/0!	#DIV/0!	#DIV/0!	1.9	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.855	#DIV/0!	#DIV/0!	#DIV/0!	1.9	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.832	#DIV/0!	#DIV/0!	#DIV/0!	1.848889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.832	#DIV/0!	#DIV/0!	#DIV/0!	1.848889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.8005	#DIV/0!	#DIV/0!	#DIV/0!	1.778889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.8005	#DIV/0!	#DIV/0!	#DIV/0!	1.778889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.779	#DIV/0!	#DIV/0!	#DIV/0!	1.731111	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.779	#DIV/0!	#DIV/0!	#DIV/0!	1.731111	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.7545	#DIV/0!	#DIV/0!	#DIV/0!	1.676667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.7545	#DIV/0!	#DIV/0!	#DIV/0!	1.676667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.733	#DIV/0!	#DIV/0!	#DIV/0!	1.628889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.733	#DIV/0!	#DIV/0!	#DIV/0!	1.628889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.712	#DIV/0!	#DIV/0!	#DIV/0!	1.582222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.712	#DIV/0!	#DIV/0!	#DIV/0!	1.582222	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.6915	#DIV/0!	#DIV/0!	#DIV/0!	1.536667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.6915	#DIV/0!	#DIV/0!	#DIV/0!	1.536667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.6505	#DIV/0!	#DIV/0!	#DIV/0!	1.445556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.6505	#DIV/0!	#DIV/0!	#DIV/0!	1.445556	#DIV/0!	#DIV/0!

| Measurement data of chromosomes for *Barbodes gonionotus* |
pair	type	B1 long arm (l)	B2 long arm (l)	B3 long arm (l)	B4 long arm (l)	B5 long arm (l)	long arm (l) sum of B	long arm (l) mean of B
1	m	0.8	0.78	1.115	0.955	0.675	4.325	0.865
1	m	0.955	0.755	0.925	0.815	0.7	4.15	0.83
2	m	0.965	0.815	0.82	0.785	0.545	3.93	0.786
2	m	0.82	0.775	0.795	0.755	0.665	3.81	0.762
3	m	0.7	0.7	0.955	0.85	0.64	3.845	0.769
3	m	0.74	0.785	0.645	0.855	0.72	3.745	0.749
4	sm	1.32	1.44	1.525	1.51	1.41	7.205	1.441
4	sm	1.27	1.27	1.285	1.44	1.215	6.48	1.296
5	sm	1.185	1.44	1.31	1.505	1.04	6.48	1.296
5	sm	1.145	1.355	1.315	1.415	0.895	6.125	1.225
6	sm	1.11	1.245	1.27	1.22	1.03	5.875	1.175
6	sm	1.025	1.12	0.855	1.185	0.815	5	1
7	sm	1.03	0.99	1.045	1.04	0.815	4.92	0.984
7	sm	0.86	0.895	1.07	0.955	0.815	4.595	0.919
8	sm	1	1.05	0.86	0.935	0.87	4.715	0.943
8	sm	0.9	0.89	0.87	0.925	0.765	4.35	0.87
9	sm	0.88	0.855	0.72	0.855	0.735	4.045	0.809
9	sm	0.87	0.77	0.8	0.85	0.685	3.975	0.795
10	sm	0.915	0.715	0.885	0.9	0.875	4.29	0.858
10	sm	0.805	0.785	0.87	0.68	0.81	3.95	0.79
11	sm	0.775	0.72	0.78	0.74	0.75	3.765	0.753
11	sm	0.775	0.72	0.755	0.81	0.775	3.835	0.767
12	st	1.1	0.95	1.415	1.1	1	5.565	1.113
12	st	0.81	0.97	1.56	0.84	0.83	5.01	1.002
13	st	0.93	0.955	1.04	0.855	0.855	4.635	0.927
13	st	0.8	1	0.93	1.07	0.91	4.71	0.942
14	st	0.925	0.855	1.03	0.965	0.715	4.49	0.898
14	st	0.855	0.84	0.915	0.88	0.845	4.335	0.867
15	a	1.675	1.5	1.44	1.635	1.225	7.475	1.495
15	a	1.5	1.42	1.32	1.34	1.16	6.74	1.348
16	a	1.42	1.315	1.29	1.325	1.11	6.46	1.292
16	a	1.415	1.285	1.29	1.325	1.1	6.415	1.283

Measurement data of chromosomes for *Barbodes gonionotus*							
		B1	B2	B3	B4	B5	long arm (l)	long arm (l)
pair	type	long arm (l)	long arm (l)	long arm (l)	long arm (l)	long arm (l)	sum of B	mean of B
17	a	1.27	1.275	1.28	1.305	1.085	6.215	1.243
17	a	1.24	1.27	1.27	1.295	1.065	6.14	1.228
18	a	1.22	1.25	1.27	1.29	1.045	6.075	1.215
18	a	1.22	1.22	1.26	1.29	1.04	6.03	1.206
19	a	1.22	1.22	1.245	1.24	1.015	5.94	1.188
19	a	1.2	1.2	1.245	1.205	1	5.85	1.17
20	a	1.2	1.185	1.245	1.18	1	5.81	1.162
20	a	1.19	1.17	1.23	1.165	1	5.755	1.151
21	a	1.17	1.14	1.18	1.16	0.995	5.645	1.129
21	a	1.17	1.135	1.17	1.135	0.99	5.6	1.12
22	a	1.17	1.13	1.155	1.135	0.98	5.57	1.114
22	a	1.16	1.09	1.145	1.13	0.975	5.5	1.1
23	a	1.15	1.075	1.135	1.12	0.965	5.445	1.089
23	a	1.145	1.075	1.1	1.1	0.965	5.385	1.077
24	a	1.115	1.07	1.1	1.085	0.93	5.3	1.06
24	a	1.04	1.02	1.08	1.06	0.915	5.115	1.023
25	a	1.025	0.9	1.035	1.05	0.9	4.91	0.982
25	a	1.02	0.825	0.88	1	0.895	4.62	0.924

Measurement data of chromosomes for *Barbodes gonionotus*								
long arm (l)	B1B	B2	B3	B4	B5	short arm (s)	short arm (s)	short arm (s)
SD of B	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of B	mean of B	SD of B
0.171865	0.7	0.745	0.805	0.7	0.635	3.585	0.717	0.062909
0.108858	0.745	0.69	0.685	0.765	0.545	3.43	0.686	0.086052
0.151756	0.73	0.545	0.695	0.665	0.4	3.035	0.607	0.135028
0.05933	0.505	0.54	0.565	0.485	0.44	2.535	0.507	0.048554
0.12973	0.48	0.425	0.6	0.505	0.395	2.405	0.481	0.079483
0.077894	0.49	0.5	0.45	0.505	0.425	2.37	0.474	0.034893
0.082795	0.485	0.57	0.625	0.605	0.55	2.835	0.567	0.054383
0.084809	0.54	0.57	0.64	0.595	0.525	2.87	0.574	0.045744
0.188726	0.48	0.5	0.625	0.515	0.48	2.6	0.52	0.060519
0.21	0.45	0.5	0.625	0.535	0.415	2.525	0.505	0.081317
0.101489	0.465	0.635	0.74	0.52	0.395	2.755	0.551	0.137359
0.161632	0.385	0.605	0.49	0.49	0.34	2.31	0.462	0.103477
0.096915	0.575	0.56	0.465	0.425	0.44	2.465	0.493	0.069696
0.098704	0.5	0.52	0.49	0.4	0.41	2.32	0.464	0.055045
0.082128	0.36	0.445	0.35	0.485	0.39	2.03	0.406	0.057598
0.061948	0.375	0.41	0.34	0.51	0.355	1.99	0.398	0.067879
0.075283	0.435	0.445	0.385	0.5	0.38	2.145	0.429	0.049168
0.073144	0.4	0.445	0.445	0.455	0.38	2.125	0.425	0.032977
0.081363	0.37	0.355	0.38	0.43	0.31	1.845	0.369	0.043359
0.069192	0.33	0.45	0.385	0.36	0.285	1.81	0.362	0.061705
0.0249	0.3	0.315	0.38	0.3	0.33	1.625	0.325	0.033166
0.032901	0.41	0.3	0.38	0.305	0.3	1.695	0.339	0.052249
0.180887	0.355	0.255	0.465	0.27	0.3	1.645	0.329	0.085103
0.318229	0.25	0.315	0.505	0.21	0.22	1.5	0.3	0.121707
0.077347	0.19	0.23	0.27	0.18	0.195	1.065	0.213	0.037014
0.101341	0.245	0.18	0.305	0.21	0.225	1.165	0.233	0.046717
0.120395	0.17	0.265	0.22	0.275	0.145	1.075	0.215	0.057118
0.030943	0.17	0.215	0.21	0.26	0.2	1.055	0.211	0.032481
0.17885						0	#DIV/0!	#DIV/0!
0.126965						0	#DIV/0!	#DIV/0!
0.11306						0	#DIV/0!	#DIV/0!
0.114815						0	#DIV/0!	#DIV/0!

Measurement data of chromosomes for *Barbodes gonionotus*								
long arm (l)	B1B	B2	B3	B4	B5	short arm (s)	short arm (s)	short arm (s)
SD of B	short arm (s)	short arm (s)	short arm (s)	short arm (s)	short arm (s)	sum of B	mean of B	SD of B
0.089345						0	#DIV/0!	#DIV/0!
0.09318						0	#DIV/0!	#DIV/0!
0.098489						0	#DIV/0!	#DIV/0!
0.097365						0	#DIV/0!	#DIV/0!
0.097378						0	#DIV/0!	#DIV/0!
0.096889						0	#DIV/0!	#DIV/0!
0.094114						0	#DIV/0!	#DIV/0!
0.088204						0	#DIV/0!	#DIV/0!
0.076354						0	#DIV/0!	#DIV/0!
0.07475						0	#DIV/0!	#DIV/0!
0.0766						0	#DIV/0!	#DIV/0!
0.074582						0	#DIV/0!	#DIV/0!
0.074783						0	#DIV/0!	#DIV/0!
0.067509						0	#DIV/0!	#DIV/0!
0.074582						0	#DIV/0!	#DIV/0!
0.064382						0	#DIV/0!	#DIV/0!
0.075382						0	#DIV/0!	#DIV/0!
0.083021						0	#DIV/0!	#DIV/0!

Measurement data of chromosomes for *Barbodes gonionotus*								
l+s	l/s	long arm	short arm	l+s	l/s	adjust	adjust	adjust
mean of B	mean of B	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)
1.582	1.206416	0.8475	0.7015	1.549	1.208164	1.883333	1.558889	3.442222
1.516	1.209913	0.8475	0.7015	1.549	1.208164	1.883333	1.558889	3.442222
1.393	1.294893	0.774	0.557	1.331	1.398926	1.72	1.237778	2.957778
1.269	1.502959	0.774	0.557	1.331	1.398926	1.72	1.237778	2.957778
1.25	1.598753	0.759	0.4775	1.2365	1.589461	1.686667	1.061111	2.747778
1.223	1.580169	0.759	0.4775	1.2365	1.589461	1.686667	1.061111	2.747778
2.008	2.541446	1.3685	0.5705	1.939	2.399643	3.041111	1.267778	4.308889
1.87	2.25784	1.3685	0.5705	1.939	2.399643	3.041111	1.267778	4.308889
1.816	2.492308	1.2605	0.5125	1.773	2.459025	2.801111	1.138889	3.94
1.73	2.425743	1.2605	0.5125	1.773	2.459025	2.801111	1.138889	3.94
1.726	2.132486	1.0875	0.5065	1.594	2.148494	2.416667	1.125556	3.542222
1.462	2.164502	1.0875	0.5065	1.594	2.148494	2.416667	1.125556	3.542222
1.477	1.995943	0.9515	0.4785	1.43	1.988273	2.114444	1.063333	3.177778
1.383	1.980603	0.9515	0.4785	1.43	1.988273	2.114444	1.063333	3.177778
1.349	2.32266	0.9065	0.402	1.3085	2.254295	2.014444	0.893333	2.907778
1.268	2.18593	0.9065	0.402	1.3085	2.254295	2.014444	0.893333	2.907778
1.238	1.885781	0.802	0.427	1.229	1.878185	1.782222	0.948889	2.731111
1.22	1.870588	0.802	0.427	1.229	1.878185	1.782222	0.948889	2.731111
1.227	2.325203	0.824	0.3655	1.1895	2.253762	1.831111	0.812222	2.643333
1.152	2.18232	0.824	0.3655	1.1895	2.253762	1.831111	0.812222	2.643333
1.078	2.316923	0.76	0.332	1.092	2.28973	1.688889	0.737778	2.426667
1.106	2.262537	0.76	0.332	1.092	2.28973	1.688889	0.737778	2.426667
1.442	3.382979	1.0575	0.3145	1.372	3.361489	2.35	0.698889	3.048889
1.302	3.34	1.0575	0.3145	1.372	3.361489	2.35	0.698889	3.048889
1.14	4.352113	0.9345	0.223	1.1575	4.197516	2.076667	0.495556	2.572222
1.175	4.042918	0.9345	0.223	1.1575	4.197516	2.076667	0.495556	2.572222
1.113	4.176744	0.8825	0.213	1.0955	4.142874	1.961111	0.473333	2.434444
1.078	4.109005	0.8825	0.213	1.0955	4.142874	1.961111	0.473333	2.434444
#DIV/0!	#DIV/0!	1.4215	#DIV/0!	#DIV/0!	#DIV/0!	3.158889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.4215	#DIV/0!	#DIV/0!	#DIV/0!	3.158889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.2875	#DIV/0!	#DIV/0!	#DIV/0!	2.861111	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.2875	#DIV/0!	#DIV/0!	#DIV/0!	2.861111	#DIV/0!	#DIV/0!

Measurement data of chromosomes for *Barbodes gonionotus*								
l+s	l/s	long arm	short arm	l+s	l/s	adjust	adjust	adjust
mean of B	mean of B	pair mean	pair mean	pair mean	pair mean	l(micron)	s(micron)	l+s(micron)
#DIV/0!	#DIV/0!	1.2355	#DIV/0!	#DIV/0!	#DIV/0!	2.745556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.2355	#DIV/0!	#DIV/0!	#DIV/0!	2.745556	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.2105	#DIV/0!	#DIV/0!	#DIV/0!	2.69	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.2105	#DIV/0!	#DIV/0!	#DIV/0!	2.69	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.179	#DIV/0!	#DIV/0!	#DIV/0!	2.62	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.179	#DIV/0!	#DIV/0!	#DIV/0!	2.62	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.1565	#DIV/0!	#DIV/0!	#DIV/0!	2.57	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.1565	#DIV/0!	#DIV/0!	#DIV/0!	2.57	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.1245	#DIV/0!	#DIV/0!	#DIV/0!	2.498889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.1245	#DIV/0!	#DIV/0!	#DIV/0!	2.498889	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.107	#DIV/0!	#DIV/0!	#DIV/0!	2.46	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.107	#DIV/0!	#DIV/0!	#DIV/0!	2.46	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.083	#DIV/0!	#DIV/0!	#DIV/0!	2.406667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.083	#DIV/0!	#DIV/0!	#DIV/0!	2.406667	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0415	#DIV/0!	#DIV/0!	#DIV/0!	2.314444	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	1.0415	#DIV/0!	#DIV/0!	#DIV/0!	2.314444	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.953	#DIV/0!	#DIV/0!	#DIV/0!	2.117778	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	0.953	#DIV/0!	#DIV/0!	#DIV/0!	2.117778	#DIV/0!	#DIV/0!

Biography

Miss Chantima Piyapong was born on the 8th of November 1974 in Chachoengsao Province, Thailand. She obtained her bachelor degree of science, in Zoology from the Faculty of Science, Chulalongkorn University in 1995. She continued for her master degree in Zoology at the same Faculty in 1996. In the first academic year, she was awarded a scholarship from the Professor Dr. Tab Nilanidhi Foundation(The Tab Foundation, Faculty of Science, Chulalongkorn University) and working as a teacher assistant(TA). In the second and the third academic years(1997-1999), she was awarded a scholarship from the University Development Committee (UDC), Ministry of University Affairs, Bangkok. Her plan is being a full-time lecturer at the Department of Biology, Faculty of Science, Mahasarakam University.