

Comparative karyotype study of three Cyprinids (Cyprinidae, Cyprininae) in Thailand by classical cytogenetic and FISH techniques

Sumalee Phimphan¹, Patcharaporn Chaiyasan²,
Chatmongkon Suwannapoom³, Montri Reungsing⁴,
Sippakorn Juntaree⁵, Alongklod Tanomtong², Weerayuth Supiwong⁵

1 Biology program, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun 67000, Thailand **2** Toxic Substances in Livestock and Aquatic Animals Research Group, Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen 40002, Thailand **3** Department of Fishery, School of Agriculture and Natural Resources, University of Phayao, Muang, Phayao 56000, Thailand **4** Department of Biotechnology, Faculty of Science and Technology, Rajamangala University of Technology Tawan-ok, Sri Racha, Chon Buri 20110, Thailand **5** Faculty of Interdisciplinary Studies, Nong Khai Campus, Khon Kaen, University, Muang, Nong Khai 43000, Thailand

Corresponding author: Weerayuth Supiwong (supiwong@hotmail.com)

Academic editor: I. Bakloushinskaya | Received 18 May 2020 | Accepted 27 June 2020 | Published 22 December 2020

<http://zoobank.org/642B0F7E-6472-462C-BEC3-8EE99C322421>

Citation: Phimphan S, Chaiyasan P, Suwannapoom C, Reungsing M, Juntaree S, Tanomtong A, Supiwong W (2020) Comparative karyotype study of three Cyprinids (Cyprinidae, Cyprininae) in Thailand by classical cytogenetic and FISH techniques. *Comparative Cytogenetics* 14(4): 597–612. <https://doi.org/10.3897/CompCytogen.v14i4.54428>

Abstract

Three species of ornamental fishes in the subfamily Cyprininae (family Cyprinidae) namely, *Epalzeorhynchus frenatum* (Fowler, 1934), *Puntigrus partipentazona* (Fowler, 1934), *Scaphognathops bandanensis* Boonyaratpalin et Srirungroj, 1971 were studied by classical cytogenetic and fluorescent in situ hybridization (FISH) techniques. Chromosomes were directly prepared from kidney tissues and stained by using conventional and Ag-NOR banding techniques. Microsatellite d(CA)₁₅ and d(CGG)₁₀ probes were hybridized to the chromosomes of three cyprinids. The results show that the three cyprinid species share the same diploid number as 2n=50 but there are differences in the fundamental number (NF) and karyotypes i.e. *E. frenatum*: NF = 78, 18m+10sm+10st+12a; *P. partipentazona*: NF = 80, 6m+24sm+14st+6a; *S. bandanensis*: NF = 66, 4m+12sm+34a. NOR positive masks were observed at the regions adjacent to the telomere of the short arm of the chromosome pairs 10 (submetacentric) and 1 (metacentric) in *E. frenatum* and *P. partipentazona*, respectively whereas those were revealed at telomeric regions of the long arm of the chromosome pair 9 (acrocentric) in *S. bandanensis*. The mapping of d(CA)₁₅ and d(CGG)₁₀

microsatellites shown that hybridization signals are abundantly distributed in telomeric regions of several pairs except d(CA)₁₅ repeats in *S. bandanensis*, which are distributed throughout all chromosomes and d(CGG)₁₀ repeats in *P. partipentazona* display the high accumulation only in the first chromosome pair.

Keywords

Chromosome, *Epalzeorhynchus frenatum*, FISH, *Puntigrus partipentazona*, *Scaphognathops bandanensis*

Introduction

There are about 200 species of freshwater fish used as ornamentals in Thailand. More than half of all ornamental fishes in Thailand belong to the family Cyprinidae. The most popular species include *Betta splendens* Regan, 1910, *Gyrinocheilus aymonieri* (Tirant, 1883), *Epalzeorhynchus bicolor* (Smith, 1931), *E. frenatum* (Fowler, 1934), *Puntigrus tetrazona* (Bleeker, 1855), *Channa micropeltes* (Cuvier, 1831), *Barbonymus alter* Bleeker, 1853, *Bar. schwanenfeldii* (Bleeker, 1854) and *Balantiocheilus melanopterus* (Bleeker, 1850) (Sermwatanakul 2005).

Family Cyprinidae is the most abundant and globally widespread family of freshwater fish, comprising 3,000 extant and extinct species in about 370 genera (Eschmeyer et al. 2015). The subfamily Cyprininae is one of the largest groups of this family. The essential large tribes such as Labeonini, Poropuntiini and Smiliogastrini have many species that are economically important ornamental fish of Thailand, namely *Epalzeorhynchus frenatum* (Fowler, 1934), *Puntigrus partipentazona* (Fowler, 1934), *Scaphognathops bandanensis* Boonyaratpalin et Srirungroj, 1971 (Fig. 1A, D, G). However, there are few studies of cytogenetics of these ornamental fishes. To date, most reports are of conventional technique studies to determine chromosome number and karyotype composition and only a few include NOR banding analysis. The 2n ranges from 48–50 in the tribes Labeonini and Smiliogastrini while the tribe Poropuntiini is more conserved as 2n = 50 (Arai 2011) (Table 1). Understanding of the basic information on cytogenetics can be applied to the development of potentially commercial stains/species in the future. The studies on the karyotypes help to investigate the genetic structure of aquatic animal species in each habitat, thus it can determine what species are related to each other in an accurate manner. This may help to facilitate the hybridization between them in the future for strain improvement (Sofy et al. 2008), breeding practices of organisms by using chromosome set management (Na-Nakorn et al. 1980), brood stock selection (Mengampan et al. 2004).

For some species, the simple characterization of the karyotype may be sufficient to identify intra- and inter-specific variants. However, in most cases, just the karyotype description appears to be inconclusive when not coupled with other methods capable of generating more accurate chromosomal markers. In this sense, the use of molecular cytogenetic analyses has played an important role in the precise characterization of the structure of genomes (Cioffi and Bertollo 2012). Multiple DNA copies or repetitive DNAs are a large substantial portion of the genome of eukaryotes that can be generally classified into two main classes: tandem repeats, such as the multigene families and the

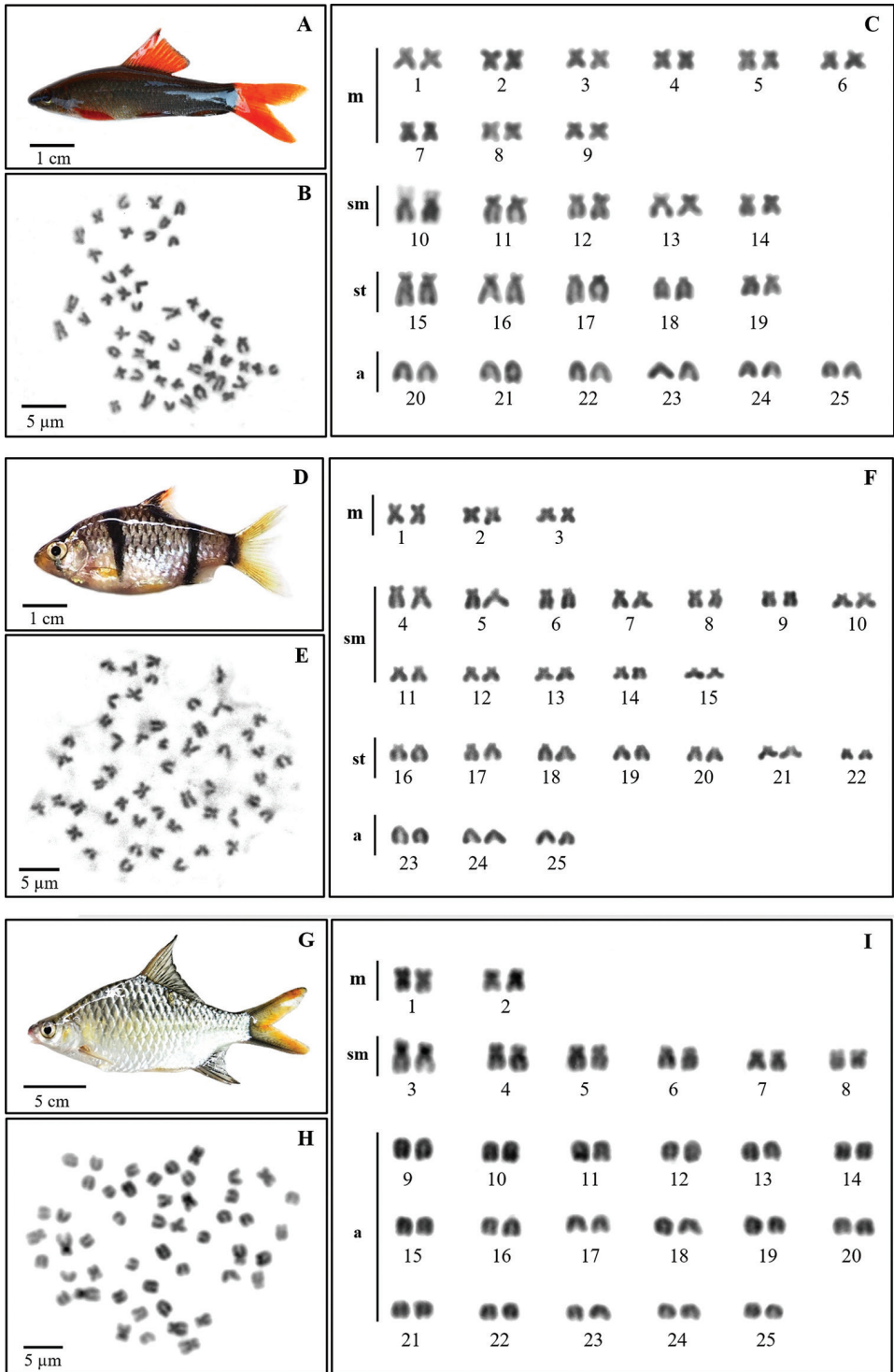


Figure 1. Specimens, metaphase chromosome plates and karyotypes of *Epalzeorhynchus frenatum* (A–C), *Puntigrus partipentazona* (D–F), *Scaphognathops bandanensis* (G–I) by conventional technique.

Table 1. Reviews of cytogenetic reports in the tribes Labeonini, Poropuntiini, and Smiliogastrini. (2n = diploid number, m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric and NORs = nucleolar organizer regions, NF = fundamental number, – = not available).

Tribe / Genus / Species	2n	NF	Formula	NORs	Reference
Tribe Labeonini					
<i>Barbichthys laevis</i> (Valenciennes, 1842)	50	76	20m+6sm+4st+20a	–	Donsakul et al. (2006)
<i>Bangana devdevi</i> (Hora, 1936)	50	86	20m+16sm+14a	–	Donsakul et al. (2011)
<i>Cirrhinus julleini</i> (Valenciennes, 1844)	50	90	26m+14sm+4st+6a	–	Magtoon and Arai (1993)
<i>C. microlepis</i> Sauvage, 1878	50	92	36m+6sm+2st+6a	–	Donsakul (1997)
	50	88	22m+8sm+8st+12a	–	Donsakul and Magtoon (1997)
	50	72	12m+10sm+2st+26a	–	Donsakul et al. (2007)
<i>Epalzeorhynchus frenatum</i> (Fowler, 1934)	48	72	14m+10sm+8st+16a	–	Donsakul and Magtoon (1993)
	50	78	18m+10sm+10st+12a	2	Present study
<i>E. bicolor</i> (Smith, 1931)	50	74	20m+4sm+2st+24a	–	Donsakul and Magtoon (1993)
<i>E. munensis</i> (Smith, 1934)	50	84	22m+12sm+2st+14a	–	Donsakul et al. (2012)
<i>Garra cambodgiensis</i> (Tirant, 1883)	50	82	20m+12sm+4st+14t	–	Donsakul et al. (2016)
<i>G. fasciacauda</i> Fowler, 1937	50	84	18m+14sm+2st+16t	–	Donsakul et al. (2016)
<i>G. notata</i> (Blyth, 1860)	50	80	20m+10sm+20t	–	Donsakul et al. (2016)
<i>Incisilabeo behri</i> (Fowler, 1937)	50	78	12m+16sm+4st+18t	–	Donsakul and Magtoon (2003)
<i>Labeo chrysophekadian</i> (Bleeker, 1850)	50	78	4m+10sm+14st+22a	–	Setapan (2007)
<i>Labiobarbus lineatus</i> (Sauvage, 1878)	50	80	20m+10sm+20a	–	Magtoon and Arai (1990)
<i>L. spiropleura</i> (Sauvage, 1881)	50	90	34m+4sm+2st+10a	–	Donsakul and Magtoon (1997)
<i>Mekongina erythrospila</i> Fowler, 1937	50	74	10m+14sm+26a(t)	–	Donsakul and Magtoon (2003)
<i>Osteochilus melanopleura</i> (Bleeker, 1852)	50	96	36m+10sm+2st+2a	–	Donsakul and Magtoon (1995)
<i>O. microcephalus</i> (Valenciennes, 1842)	50	86	26m+10sm+14st	–	Donsakul et al. (2001)
<i>O. vittatus</i> (Valenciennes, 1842)	50	96	16m+30sm+4st	–	Magtoon and Arai (1990)
	50	86	26m+10sm+14st	–	Donsakul (1997)
<i>O. waandersi</i> (Bleeker, 1853)	50	92	18m+24sm+4st+4a	2	Magtoon and Arai (1993)
<i>Puntioplites falcifer</i> Smith, 1929	50	80	14m+16sm+2st+18a	–	Donsakul et al. (2007)
	50	92	16m+10sm+16a+8t	–	Sophawanus et al. (2017)
Tribe Smiliogastrini					
<i>Osteobrama alfrediana</i> (Valenciennes, 1844)	50	96	24m+22sm+4a	–	Donsakul et al. (2011)
<i>Hampala disper</i> Smith, 1934	50	70	5m+5sm+3st+12a	–	Donsakul and Poopitayasathaporn (2002)
<i>H. macrolepidota</i> Kuhl & Van Hasselt, 1823	50	72	10m+12sm+8st+20a	–	Donsakul and Poopitayasathaporn (2002)
<i>Puntigrus partipentazona</i> (Fowler, 1934)	50	90	6m+34sm+10a	–	Taki et al. (1977)
	50	80	6m+24sm+14st+6a	2	Present study
<i>P. tetrazona</i> (Bleeker, 1855)	50	84	34m+6st+10a	–	Ohno et al. (1967)
	50	84	6m+28sm+16a	–	Hinegardner and Rosen (1972), Taki et al. (1977), Suzuki et al. (1995)
	50	–	–	–	Krishnaja and Rege (1980) Vinogradov (1998)
<i>P. tetrazona partipentazona</i> (Fowler, 1937)	50	90	6m+34sm+10a	–	Taki et al. (1977)
<i>Puntius arulius</i> (Jerdon, 1849)	50	82	6m+26sm+18a	–	Taki and Suzuki (1977)
	50	90	10m+18sm+12st+10t	–	Arunachalan and Murugan (2007)
<i>P. binotatus</i> (Valenciennes, 1842)	50	92	8m+34sm+8a	–	Taki et al. (1977)
<i>P. brevis</i> (Bleeker, 1850)	50	70	6m+14sm+8st+22a	–	Khuda-Bukhsh (1975)
	50	54	2m+2sm+2st+22a	–	Donsakul and Poopitayasathaporn (2002)
	48	56	2m+6st+40a	–	Setapan (2007)
	50	62	4m+4sm+4a+38t	2	Nitikulworawong and Khruaeant (2014)
<i>P. cholai</i> (Hamilton, 1822)	50	56	2m+4sm+44a	–	Taki and Suzuki (1977)
	50	54	2m+2sm+4st+42a	–	Tripathi and Sharma (1987)
	50	54	2m+2sm+46a	–	Sahoo et al. (2007)
<i>P. conchoniui</i> (Hamilton, 1822)	50	94	6m+38sm+6a	–	Hinegardner and Rosen (1972), Taki and Suzuki (1977)
	48	78	10m+20sm+10st+8a	–	Sharma and Agarwal (1981)
	50	–	–	–	Vasiliev (1985)
	50	90	16m+24sm+2st+8a	–	Khuda et al. (1986), Ojima and Yamamoto (1990)

Tribe / Genus / Species	2n	NF	Formula	NORs	Reference
<i>P. conchoni</i> (Hamilton, 1822)	50	94	4m+40sm+6a	–	Taki and Ojima (1988)
<i>P. cumingi</i> (Günther, 1868)	50	94	18m+26sm+6a	–	Taki and Suzuki (1977)
<i>P. daruphani</i> Smith, 1934	50	70	12m+8sm+6st+24a	–	Magtoon and Arai (1989)
<i>P. denisonii</i> (Day, 1865)	50	74	4m+20sm+18st+8a	8	Nagpure et al. (2004)
<i>P. everetti</i> (Boulenger, 1894)	50	86	6m+30sm+14a	–	Hinegardner and Rosen (1972), Taki et al. (1977), Vinogradov (1998)
<i>P. fasciatus</i> (Jerdon, 1849)	50	80	30m+4st+16a	–	Ohno et al. (1967)
	50	82	6m+26sm+18a	–	Taki et al. (1977)
<i>P. filamentosus</i> (Valenciennes, 1844)	50	84	8m+26sm+16a	–	Taki and Suzuki (1977)
	50	78	12m+16sm+12st+10a	8	Nagpure et al. (2003)
<i>P. lateristriga</i> (Valenciennes, 1842)	50	88	6m+32sm+12a	–	Taki et al. (1977)
	50	86	22m+14sm+6st+8a	–	Sobita et al. (2004)
<i>P. melanampyx</i> Day, 1865	50	74	12m+12sm+14st+12a	–	Khuda et al. (1986)
<i>P. nigrofasciatus</i> (Günther, 1868)	50	100	16m+34sm	–	Taki and Suzuki (1977)
<i>P. oligolepis</i> (Bleeker, 1853)	50	88	8m+30sm+12a	–	Taki et al. (1977)
	50	80	14m+16sm+4st+16a	–	Arai and Magtoon (1991)
	50	92	6m+36sm+8a	–	Taki et al. (1977)
<i>P. pentazona</i> (Boulenger, 1894)	50	98	22m+26sm+2a	–	Taki et al. (1977)
<i>P. sarana</i> (Hamilton, 1822)	50	76	12m+14sm+12st+12a	–	Rishi (1981)
<i>P. sarana subnasutus</i> (Valenciennes, 1842)	50	88	12m+26sm+8st+4a	–	Nagpure et al. (2004)
<i>P. semifasciolatus</i> (Günther, 1868)	50	76	12m+14sm+14st+10a	–	Gui et al. (1986), Yu et al. (1989)
	50	76	12m+14sm+14st+10a	8	Nagpure et al. (2004)
	50	76	8m+18sm+24a	–	Suzuki (1991)
<i>P. sophore</i> (Hamilton, 1822)	48	52	2m+2sm+44a	–	Rishi (1973)
	48	54	2m+4sm+42a	–	Rishi et al. (1977)
	48	52	4m+2st+42a	–	Rishi and Rishi (1981)
	50	56	2m+4sm+44a	–	Khuda et al. (1986)
	48	52	4m+6st+38a	–	Tripathi and Sharma (1987)
<i>P. sophoroides</i> (Günther, 1868)	50	54	2m+2sm+46a	–	Magtoon and Arai (1989)
<i>P. stoliczkanus</i> (Day, 1871)	50	94	22m+22sm+4st+2a	–	Magtoon and Arai (1989)
<i>P. tamraparniei</i> Silas, 1954	50	94	12m+16sm+16a+6t	–	Arunachalan and Murugan (2007)
<i>P. ticto</i> (Hamilton, 1822)	50	82	20m+12sm+10st+8a	–	Sharma et al. (1995), Vinogradov (1998)
	50	100	28m+22sm	–	Taki and Suzuki (1977)
	50	94	28m+16sm+6st	–	Sahoo et al. (2007)
<i>P. titteya</i> (Deraniyagala, 1929)	50	98	20m+28sm+2a	–	Hinegardner and Rosen (1972), Taki and Suzuki (1977)
	48	52	4m+2sm+42a	–	Khuda-Bukhsh and Barat (1987)
<i>Systomus</i> sp.1	50	82	12m+20sm+6st+12a	–	Donsakul et al. (2006)
<i>S. binotatus</i> (Valenciennes, 1842)	50	88	24m+14sm+12a	–	Donsakul and Magtoon (2002)
<i>S. orphoides</i> (Valenciennes, 1842)	50	82	12m+20sm+4st+14a	–	Piyapong (1999)
	50	74	8m+16sm+10st+16a	–	Donsakul and Poopitayasathaporn (2002)
<i>S. stoliczkanus</i> (Day, 1871)	50	94	24m+20sm+6a	–	Donsakul et al. (2011)
Tribe Poropuntiini					
<i>Amblyrhynchichthys truncatus</i> (Bleeker, 1851)	50	78	16m+12sm+22a	–	Donsakul et al. (2006)
<i>Balantiocheilus melanopterus</i> (Bleeker, 1850)	50	72	10m+12sm+28a	–	Ojima and Yamamoto (1990)
	50	70	14m+6sm+10st+20a	–	Donsakul and Poopitayasathaporn (2002)
<i>Barbonyms gonionotus</i> (Bleeker, 1850)	50	72	2m+20sm+4st+24a	–	Magtoon and Arai (1989)
	50	74	16m+8sm+26a	–	Donsakul and Magtoon (1997)
	50	72	6m+16sm+6st+22a	–	Piyapong (1999)
	50	66	2m+4sm+10st+34a	–	Seetapan (2007)
	50	74	6m+18sm+16st+10a	2	Khuda-Bukhsh and Das (2007)
<i>Cosmochilus harmandi</i> Sauvage, 1878	50	82	22m+10sm+10st+8a	–	Donsakul et al. (2005)
<i>Cyclocheilichthys apogon</i> (Valenciennes, 1842)	50	70	12m+8sm+6st+24a	–	Magtoon and Arai (1989)
	50	76	18m+8sm+4st+20a	–	Donsakul and Poopitayasathaporn (2002)
	50	86	10m+16sm+10a+14t	6	Chantapan (2015)
<i>C. lagleri</i> Sontirat, 1989	50	86	12m+6sm+1st+6a	–	Donsakul et al. (2006)
<i>C. repasson</i> (Bleeker, 1851)	50	78	12m+16sm+6st+16a	–	Donsakul et al. (2005)
	50	84	6m+6sm+22st+16a	–	Seetapan (2007)

Tribe / Genus / Species	2n	NF	Formula	NORs	Reference
<i>Cyclocheilichthys enoplos</i> (Bleeker, 1849)	50	90	10m+30sm+4st+6a	4	Magtoon and Arai (1993)
	50	72	14m+8sm+10st+18a	–	Donsakul and Magtoon (1995a)
	50	78	16m+12sm+6st+16a	–	Donsakul and Poopitayasathaporn (2002)
<i>Hypsibarbus lagleri</i> Rainboth, 1996	50	74	4m+20sm+26a	–	Donsakul and Magtoon (2001)
<i>H. malcolmi</i> (Smith, 1945)	50	64	10m+4sm+36a	–	Donsakul et al. (2007)
<i>H. vernayi</i> (Norman, 1925)	50	58	6m+2sm+4st+38a	–	Donsakul and Magtoon (2002)
<i>H. wetmorei</i> (Smith, 1931)	50	70	12m+8sm+6st+24a	–	Magtoon and Arai (1989)
	50	74	12m+12sm+4st+22a	2	Piyapong (1999)
	50	74	12m+12sm+2st+24a	–	Donsakul and Magtoon (2002)
<i>Mystacoleucus argenteus</i> (Day, 1888)	50	82	10m+14sm+8a+18t	6	Chantapan (2015)
	50	76	6m+20sm+2st+22a	–	Donsakul et al. (2006)
<i>M. marginatus</i> (Valenciennes, 1842)	50	76	16m+10sm+24a	–	Arai and Magtoon (1991)
	50	68	14m+4sm+2st+30a	–	Donsakul and Poopitayasathaporn (2002)
	50	74	14m+10sm+26t	–	Donsakul et al. (2005)
<i>Poropuntius deauratus</i> (Valenciennes, 1842)	50	82	10m+22sm+18st	–	Zen et al. (1984)
<i>P. sinensis</i> (Bleeker, 1871)	50	82	10m+22sm+18st	–	Zen et al. (1984)
<i>P. laoensis</i> (Günther, 1868)	50	74	14m+10sm+10st+16a	–	Donsakul and Magtoon (2008)
<i>P. normani</i> Smith, 1931	50	72	10m+12sm+28a	–	Donsakul et al. (2007)
<i>P. chonglingchungii</i> (Tchang, 1938)	50	80	12m+18sm+20st	–	Zen et al. (1986)
<i>Scaphognathops bandanensis</i> Boonyaratpalin & Srirungroj, 1971	50	66	10m+6sm+34a	–	Donsakul et al. (2007)
	50	66	10m+6sm+34a	2	Present study
<i>Sikukia gudgeri</i> (Smith, 1934)	50	68	10m+8sm+4st+28a	–	Donsakul et al. (2005)

satellite DNAs; and the dispersed elements, such as transposons and retrotransposons, known as Transposable elements (TEs) (Jurka et al. 2005). Among the tandem repeats we can find the highly-repeated satellite DNAs and “moderate repeats”, like mini- and microsatellite DNA (Charlesworth et al. 1994). These non-coding DNA sequences are organized as long arrays of head-to-tail linked repeats (Plohl et al. 2008).

Recently, the molecular cytogenetic studies using fluorescence *in situ* hybridization (FISH) for mapping repetitive DNA sequences have provided important contributions to the characterization of the biodiversity and the evolution of divergent fish groups (Cioffi and Bertollo 2012). Moreover, some microsatellite repeats are species-specific characters among some fish group (Cioffi et al. 2015). Most molecular cytogenetic studies in cypinid fishes were performed by FISH technique using rDNA probes (Inafuku et al. 2000; Kikuma et al. 2000; Ocalewicz et al. 2004; Zhu et al. 2006; Singh et al. 2009; Rossi et al. 2012; Nabais et al. 2013; Kirtiklis et al. 2014; Spoz et al. 2014; Han et al. 2015; Kumar et al. 2016; Han et al. 2017). However, NOR banding including fluorescence *in situ* hybridization (FISH) techniques to investigate chromosomal distribution of repetitive DNA sequences on the chromosomes of *E. frenatum*, *P. partipentazona*, *S. bandanensis* have not been performed.

In present study, we carried out an analysis of chromosomal structures and genetic markers on *E. frenatum*, *P. partipentazona*, and *S. bandanensis* using cytogenetics, and molecular cytogenetics techniques. The knowledge revealed will provide a powerful tool for the next generation of genome research in Thai freshwater fishes and discovering biodiversity, with useful applications in fish breeding for conservation and commercials of ornamental species. Moreover, it is useful applications in

evolution, systematics, phylogenetics, fish fauna management and suitable conservation of river basin.

Material and methods

Ten males and ten females of each species including *E. frenatum*, *P. partipentazona*, *S. bandanensis*, were collected from the Song Kham, Chi and Mekong Basins, respectively. Preparation of fish chromosomes was from kidney cells (Pinthong et al. 2015; Supiwong et al. 2015). The chromosomes were stained with Giemsa's solution for 10 min. Ag-NOR banding was performed by applying two drops of 2% gelatin on the slides, followed with four drops of 50% silver nitrate (Howell and Black 1980). Metaphase figures were analyzed according to the chromosome classification of Levan et al. (1964). Chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) or acrocentric (a). Fundamental number, NF (number of chromosome arm) is obtained by assigning a value of two to metacentric and submetacentric chromosomes and one to subtelocentric and acrocentric chromosomes.

The use of microsatellite d(CA)₁₅ and d(CGG)₁₀ probes described by Kubat et al. (2008) was followed here with slight modifications. These sequences were directly labeled with Cy3 at 5' terminal during synthesis by Sigma (St. Louis, MO, USA). FISH was performed under high stringency conditions on mitotic chromosome spreads (Pinkel et al. 1986). After denaturation of chromosomal DNA in 70% formamide/ 2×SSC at 70 °C, spreads were incubated in 2×SSC for 4 min at 70 °C. The hybridization mixture (2.5 ng/μL probes, 2 μg/μL salmon sperm DNA, 50% deionized formamide, 10% dextran sulfate) was dropped on the slides, and the hybridization was performed overnight at 37 °C in a moist chamber containing 2×SSC. The post hybridization wash was carried out with 1×SSC for 5 min at 65 °C. A final wash was performed at room temperature in 4×SSC for 5 min. Finally, the slides were counterstained with DAPI and mounted in an antifade solution (Vectashield from Vector laboratories) and analyzed in an epifluorescence microscope Olympus BX50 (Olympus Corporation, Ishikawa, Japan).

Results

Diploid number, fundamental number and karyotype of *Epalzeorhynchus frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

Results have shown that the three cyprinid species have the same diploid number of $2n = 50$. Although the three species analyzed share the same $2n$, there are differences in the fundamental number (NF) and karyotypes i.e. *E. frenatum*: NF = 78, 18 metacentric (m), 10 submetacentric (sm), 10 subtelocentric (st) and 12 acrocentric

(a) chromosomes; *P. partipentazona*: NF = 80, 6m, 24sm, 14st, and 6a chromosomes; *S. bandanensis*: NF = 66, 4m, 12sm, and 34a chromosomes (Fig. 1).

Chromosome marker of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

NOR positive masks were observed at the regions adjacent to the telomere of the short arm of the chromosome pairs 10 (submetacentric) and 1 (metacentric) in *E. frenatum* and *P. partipentazona*, respectively whereas they were revealed at telomeric regions of the long arm of the chromosome pair 9 (acrocentric) in *S. bandanensis* (Fig. 2A, D, G and Table 2).

Patterns of microsatellite repeats on the genome of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

The mapping of $d(CA)_{15}$ and $d(CGG)_{10}$ microsatellites shown that hybridization signals are abundantly distributed in telomeric regions of several pairs except $d(CA)_{15}$ repeats in *S. bandanensis*, which are distributed throughout all chromosomes and $d(CGG)_{10}$ repeats in *P. partipentazona* display the high accumulation only in the first chromosome pair. In addition, interstitial signals of $d(CA)_{15}$ and $d(CGG)_{10}$ repeats can be observed at the short arm of the chromosome pairs 3 and 4, respectively in *E. frenatum* (Fig. 2 and Table 2). Figure 3 shows the idiograms representing the patterns of $d(CA)_{15}$ and $d(CGG)_{10}$ microsatellites distributions on the chromosomes of three studied species. Microsatellite $d(CGG)_{10}$ sequences were detected disperse hybridization signals with high accumulation of them at telomeric regions of several chromosomes in *E. frenatum* and *S. bandanensis*. However, it is interesting that the microsatellite $d(CGG)_{10}$ repeats coincide with the NOR positions in *P. partipentazona*.

Discussion

Diploid number, fundamental number and karyotype of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

The diploid numbers ($2n$) are same as found in *P. partipentazona* (Taki et al. 1977) and *S. bandanensis* (Donsakul et al. 2007) but there is difference in *E. frenatum* ($2n = 48$) reported by Magtoon and Donsakul (1993). The $2n$ in three cyprinids studied have the same $2n = 50$ as in several species in the subfamily Cyprininae (Arai 2011, Table 1). It seems to be that this subfamily is highly conserved for the $2n$. To compare with the previous studies, the NF of *S. bandanensis* is same as the study of Donsakul et al. (2007) whereas ones of *E. frenatum* and *P. partipentazona* differ from the reports of

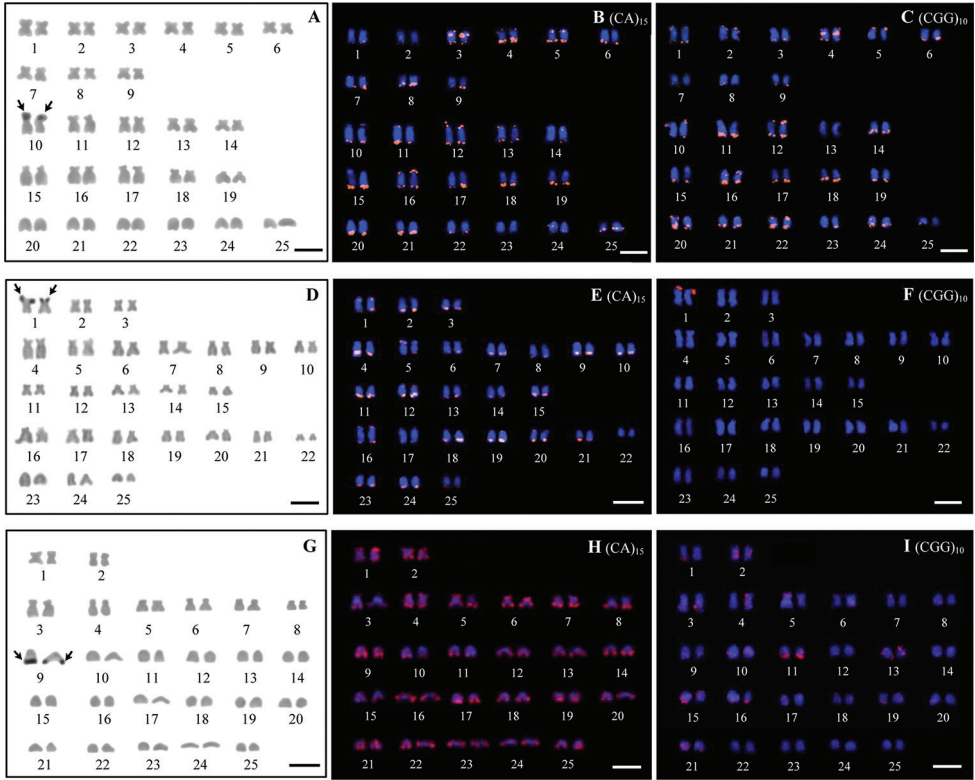


Figure 2. Karyotypes of *Epalzeorhynchus frenatum* (A–C), *Puntigrus partipentazona* (D–F), *Scaphognathops bandanensis* (G–I) by NOR banding and FISH techniques. Arrows indicate NOR-bearing chromosomes. Scale bars: 5 μm.

Table 2. Cytogenetic and FISH studies on three Cyprinid fishes in Thailand. (2n = diploid chromosome number, NF = fundamental number (number of chromosome arm), m = metacentric, sm = submetacentric, a = acrocentric, st = subtelocentric chromosomes, NOR = nucleolar organizer region).

Species	2n	NF	Chromosome type				Ag-NOR pair (type)	CA ₁₅ pair	CGG ₁₀ pair
			m	sm	st	a			
<i>E. frenatum</i>	50	84	18	10	10	12	10(sm)	1–13,15–25	1–6,9–12,14–25
<i>P. partipentazona</i>	50	94	6	24	14	6	1(m)	1–16, 18–21, 23–25	1
<i>S. bandanensis</i>	50	66	4	12	-	34	9(a)	1–25	1, 3–5,9–11, 13, 15–16, 19–21

Magtoon and Donsakul (1993) and Taki et al. (1977), respectively. The differences of NFs have cause to differences of karyotypes among these fishes. These differences may be causes from the species-specific variations among populations, and/or misidentification of species or different species due to complex species. Three studied species cannot be observed heteromorphic sex chromosomes between male and female specimens. This phenomenon is same as many species in this family (Arai 2011).

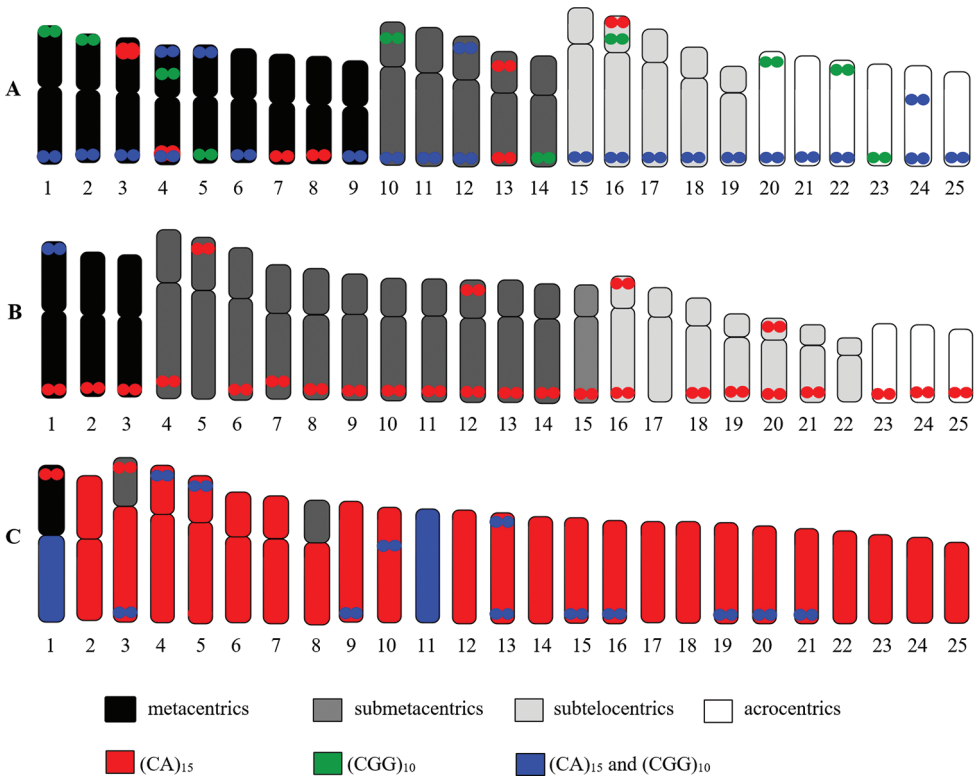


Figure 3. Idiograms represent the $(CA)_{15}$ and $(CGG)_{10}$ mapping on the chromosomes of *Epalzeorhynchus frenatum* **A** *Puntigrus partipentazona* **B** *Scaphognathops bandanensis* **C**.

Chromosome marker of *Epalzeorhynchus frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

The determination of nucleolar organizer regions (NORs) for these species was firstly proposed. If these loci are active during the interphase before to mitosis, they can be detected by silver nitrate staining (Howell and Black 1980) since they specifically stain a set of acidic proteins related to ribosomal synthesis process. The single NOR-bearing chromosome pair in the present result is consistent with results from *Barbonymus gonionotus* (Bleeker, 1849) (Khuda-Bukhsh and Das 2007), *Hypsibarbus wetmorei* (Smith, 1931) (Piyapong 1999), *Osteochilus waandersi* (Bleeker, 1853) (Magtoon and Arai 1993) and *Puntius brevis* (Bleeker, 1849) (Nitikulworawong and Khruanet 2014). This character is common characteristic found in many fish groups as well as vertebrates (Supiwong et al. 2012, 2013). However, some species had two pairs (*Cyclocheilios enoplos* (Bleeker, 1849): Magtoon and Arai 1993), three pairs (*Cyclocheilichthys apogon* (Valenciennes, 1842): Chantapan 2015) and four pairs (*Puntius denisonii* (Day, 1865), *P. semifasciolatus* (Günther, 1868): Nagpure et al. 2004; *P. filamentosus* (Valenciennes, 1844): Nagpure et al. 2003). NORs are chromosomal

landmarks that consist of tandemly repeated sequences of ribosomal genes (rRNA). In eukaryotes, each unit is composed of three genes coding for 18S, 5.8S and 28S ribosomal RNA (Sharma et al. 2002). The number and position of the rDNA clusters have been widely used in systematics and phylogenetic reconstructions since these chromosomal characters are often species-specific (Britton-Davidian et al. 2012). Changes in chromosome number and structure can alter the number, and structure of NOR. Structure, number, and morphology of a NOR may be specific to populations, species, and subspecies. Robertsonian translocations (centric fusion) may cause losses of NOR. Studies on NOR variation in numerous organism groups have invariably described changes in the number and location of NORs even in closely related species, suggesting that rDNA clusters are highly mobile components of the genome (Britton-Davidian et al. 2012). Thus, species, which have limited gene exchange due to geographical isolation, have elevated karyotype varieties and NOR variations. The use of NORs in explaining phylogenetic relationships depends on a large extent on the uniformity of this characteristic and on the degree of variety within a taxon (Yüksel and Gaffaroglu 2008). Normally, most fishes have only one pair of small NORs in a chromosome complement. If some fishes have more than two NORs, it may be caused by the translocation between NOR and another chromosome (Sharma et al. 2002).

Patterns of microsatellite repeats in the genome of *Epalzeorhynchos frenatum*, *Puntigrus partipentazona* and *Scaphognathops bandanensis*

The patterns of microsatellite $d(CA)_{15}$ in three species in the present study except in *S. bandanensis* are different from the nine species of the Bagridae family including *Hemibagrus filamentus* (Fang & Chaux, 1949), *H. spilopterus* Ng & Rainboth, 1999, *H. wyckii* (Bleeker, 1858), *H. wyckioides* Fang & Chaux, 1949, *Mystus atrifasciatus* Fowler, 1937, *M. multiradiatus* Roberts, 1992, *M. mysticetus* Roberts, 1992, *M. bocourti* (Bleeker, 1864), and *Pseudomystus siamensis* (Regan, 1913) (Supiwong et al. 2013, 2014), *Toxotes chatareus* (Hamilton, 1822) (Supiwong et al. 2017). From the previous and current studies, it may seem that all heterochromatins in fish genomes consist of microsatellites (Cioffi and Bertollo 2012). However, microsatellites have also been found in noncentromeric regions, many of them were located either near or within genes (Rao et al. 2010). This is the same as in the pattern of microsatellite $d(CGG)_{10}$ revealed in *S. bandanensis*.

Conclusions

The present research is the first report on the NOR -banding and FISH techniques in *E. frenatum*, *P. partipentazona*, *S. bandanensis*. Although all studied species have the same diploid chromosome number ($2n = 50$) and two NOR-bearing chromosomes, there are differences in the fundamental numbers, numbers of chromosomes

with equal sizes, pairs having NORs, and patterns of microsatellites distributions on chromosomes. The NORs can be observed at the regions adjacent to the telomeres of pairs 10, 1 and 9, respectively. The microsatellites are distributed throughout the chromosomes with high accumulations at some positions or all chromosomes which are species-specific characteristics. This result indicated that cytogenetic data can be used for classification in related fish species which have similar morphology.

Acknowledgments

This work was financially supported by the research grant of the Office of the Higher Education Commission and the Thailand Research Fund (MRG6080020), and the Post-Doctoral Training Program from Research Affairs and Graduate School (Grant no 59255), Khon Kaen University, and Unit of Excellence 2020 on Biodiversity and Natural Resources Management, University of Phayao (UoE63005), Thailand.

References

- Almeida-Toledo LF, Ozouf-Costaz C, Foresti F, Bonillo C, Porto-Foresti F, Daniel-Silva MFZ (2002) Conservation of the 5S-bearing chromosome pair and co-localization with major rDNA clusters in five species of *Astyanax* (Pisces, Characidae). *Cytogenet Genome Research* 97: 229–233. <https://doi.org/10.1159/000066609>
- Amemiya CT, Gold JR (1988) Chromosomal NORs as taxonomic and systematic characters in North American cyprinid fishes. *Genetica* 76: 81–90. <https://doi.org/10.1007/BF00058806>
- Arai R (2011) *Fish Karyotypes: A Check List*. Springer Japan, Tokyo. <https://doi.org/10.1007/978-4-431-53877-6>
- Arunachalan M, Murugan M (2007) Cytogenetics and cytotaxonomic consideration of two endangered ornamental fishes *Puntius arulius* and *P. tambraparniei* (Cypriniformes: Cyprinidae) from Western Ghath, India. *Zoo' Print journal* 22(7): 2739–2741. <https://doi.org/10.11609/JoTT.ZPJ.1552.2739-41>
- Britton-Davidian J, Cazaux B, Catalan J (2012) Chromosomal dynamics of nucleolar organizer regions (NORs) in the house mouse: micro-evolutionary insights. *Heredity* 108: 68–74. <https://doi.org/10.1038/hdy.2011.105>
- Chantapan T (2015) Standardized karyotype and ideogram of cyprinid fishes (Subfamily: Cyprininae) in Thailand. M.Sc. Thesis. Khon Kaen University. [In Thai]
- Charlesworth B, Sniegowski P, Stephan W (1994) The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature* 371: 215–220. <https://doi.org/10.1038/371215a0>
- Cioffi MB, Bertollo LAC (2012) Distribution and evolution of repetitive DNAs in fish. In: Garrido-Ramos MA (Ed), *Repetitive DNA*. Karger, *Genome Dyn.* 2012, vol 7, Basel, 197–221. <https://doi.org/10.1159/000337950>
- Cioffi MB, Bertollo LAC, Villa MA, Oliveira E A, Tanomtong A, Yano CF, Supiwong W, Chaveerach A (2015) Genomic organization of repetitive DNA elements and its implica-

- tions for the chromosomal evolution of channid fishes (Actinopterygii, Perciformes). PLoS ONE 12: 1–13. <https://doi.org/10.1371/journal.pone.0130199>
- Cioffi MB, Martins C, Bertollo LAC (2009) Comparative chromosome mapping of repetitive sequences. Implications for genomic evolution in the fish, *Hoplias malabaricus*. BMC Genetics 10: 1–34. <https://doi.org/10.1186/1471-2156-10-34>
- Donsakul T, Magtoon W (1995a) Karyological studies of *Puntius brevis* (Bleeker, 1850) and *Cyclocheilichthys enoplos* in Thailand. Proceeding of the 21st Congress on Science and Technology of Thailand. Queen Sirikit National Convention Centre, Bangkok, 292–293.
- Donsakul T, Magtoon W (1995b) Karyotypes of four cyprinid fishes, *Osteochilus melanopleura*, *Puntioplites proctozysron*, *Paralaubuca riveroi* and *Rasbora sumatrana*, from Thailand. Proceedings of the 33rd Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 128–138. [In Thai]
- Donsakul T, Magtoon W (1997) A chromosome study of five species of cyprinid fishes, *Cirrhina microlepis*, *Leptobarbus hoevenii*, *Labiobarbus spiroleura*, *Barbodes schwanenfeldii* and *Barbodes gonionotus* from Thailand. Srinakharinwirot Science Journal 2: 30–42.
- Donsakul T, Magtoon W (2001) Karyological studies of *Hypsibarbus lagler* and *H. vernay* in Thailand. Proceedings of the 27th National Science and Technology Development Agency, 434 pp.
- Donsakul T, Magtoon W (2002) Karyotypes of two cyprinid fishes, *Hypsibarbus wetmorei* and *Morulius chrysophekadion*, from Thailand. Proceedings of the 28th Congress on Science and Technology of Thailand. Queen Sirikit National Convention Centre, Bangkok, 92 pp.
- Donsakul T, Magtoon W (2008) Karyotypes of seven cyprinid fishes: *Systemus binotatus*, *Puntius brevis*, *Poropuntius laoensis*, *Labiobarbus siamensis*, *Catlocarpio siamensis*, *Tor tambroides* and *Probarbus jullieni* from Thailand. Srinakharinwirot Science Journal 24: 80–92.
- Donsakul T, Magtoon W, Rangsiruji A (2005) Karyotypes of four Cyprinid fishes: *Cyclocheilichthys repasson*, *Cosmochilus harmandi*, *Poropuntius deauratus* and *Sikukia gudgeri* from Thailand. Proceedings of the 43rd Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 344–351. [In Thai]
- Donsakul T, Magtoon W, Rangsiruji A (2006) Karyological studies of four Cyprinid fishes: *Barbichthys nitidus*, *Mystacoleucus argenteus*, *Cychocheilichthys lagleri* and *Systemus* sp.1 from Thailand. Proceedings of the 44rd Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 469–476. [In Thai]
- Donsakul T, Poopitayasathaporn A (2002) Karyotype of Fifteen Species of Cyprinid Fishes (Family Cyprinidae) from Thailand. Research Project. Srinakharinwirot University, Bangkok.
- Donsakul T, Rangsiruji A, Magtoon W (2007) Karyotypes of four cyprinid fishes: *Poropuntius normani*, *Hypsibarbus malcolmi*, *Scaphognathops bandanensis* and *Henicorhynchus caudiguttatus* from Thailand. The Proceedings of 45th Kasetsart University Annual Conference: Fisheries. Bangkok, 740–748. [In Thai]
- Donsakul T, Rangsiruji A, Magtoon W (2011) Karyotypes of five cyprinid fishes (Family Cyprinidae): *Bangana devdevi*, *Osteobrama alfrediana*, *Systemus stoliczkanus*, *Oxygaster anomalura* and *Aspidoparia morar* in Thailand. Proceedings of the 49th Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 169–177. [In Thai]
- Eschmeyer WN [Ed.] (2015) Catalog of Fishes: Genera, Species, References. <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> [Electronic version accessed 28 June 2016]

- Gold JR, Amemiya CT, Ellison JR (1986) Chromosomal heterochromatin differentiation in North American cyprinid fishes. *Cytologia* 51: 557–566. <https://doi.org/10.1508/cytologia.51.557>
- Gui JF, Li YC, Li K, Zhou T (1986) Studies on the karyotypes of Chinese cyprinid fishes. (VIII) Karyotypic analyses of fifteen species of Barbinae with consideration for their phyletic evolution. *Transactions of the Chinese Ichthyological Society* 5: 119–127.
- Hatanaka T, Galetti Jr PM (2004) Mapping of the 18S and 5S ribosomal RNA genes in the fish *Prochilodus argenteus* Agassiz, 1829 (Characiformes, Prochilodontidae). *Genetica* 122: 239–244. <https://doi.org/10.1007/s10709-004-2039-y>
- Hinegardner R, Rosen DE (1972) Cellular DNA content and the evolution of teleostean fishes. *The American Naturalist* 106: 621–644. <https://doi.org/10.1086/282801>
- Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014–1015. <https://doi.org/10.1007/BF01953855>
- Hsu TC, Patak S, Chen TR (1975) The possibility of latent centromeres and a proposed nomenclature system for total chromosome and whole arm translocations. *Cytogenetics Cell Genetics* 15: 41–49. <https://doi.org/10.1159/000130497>
- Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O, Walichiewicz J (2005) Repbase update, a database of eukaryotic repetitive elements. *Cytogenet Genome Research* 110: 462–467. <https://doi.org/10.1159/000084979>
- Khuda-Bukhsh AR (1975) Somatic chromosomes of an exotic fish *Puntius japonicus*. *The Journal of Cytology and Genetics (India)* 118–120. <https://doi.org/10.1007/BF00057565>
- Khuda-Bukhsh AR, Barat A (1987) Chromosomes in fifteen species of Indian teleosts (Pisces). *Caryologia* 40: 131–144. <https://doi.org/10.1080/00087114.1987.10797817>
- Khuda-Bukhsh AR, Das JK (2007) Cytogenetic analyses in eight species of teleostean fishes (Pisces): karyotypes, multiple Ag-NORs, sex chromosomes. *Research and Reviews in Biosciences (India)* 1: 47–52.
- Krishnaja AP, Rege MS (1980) Some observations on the chromosomes of certain teleosts using a simple method. *Indian Journal of Experimental Biology* 18: 268–270.
- Kubat Z, Hobza R, Vyskot B, Kejnovsky E (2008) Microsatellite accumulation in the Y chromosome of *Silene latifolia*. *Genome* 51: 350–356. <https://doi.org/10.1139/G08-024>
- Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220. <https://doi.org/10.1111/j.1601-5223.1964.tb01953.x>
- Liehr T [Ed.] (2009) *Fluorescence In Situ Hybridization (FISH)-Application Guide*. Springer, Verlag, Berlin, Heidelberg. <https://doi.org/10.1007/978-3-540-70581-9>
- Magtoon W, Arai R (1989) Karyotypes of five *Puntius* species and one *Cyclocheilichthys* species (Pisces, Cyprinidae) from Thailand. *Bulletin of the National Science Museum, Tokyo. Series A (Zoology)* 15(3): 167–175.
- Magtoon W, Arai R (1993) Karyotypes and distribution of nucleolus organizer regions in cyprinid fishes from Thailand. *Japanese Journal of Ichthyology* 40(1): 77–85. <https://doi.org/10.11369/jji1950.40.77>
- Magtoon W, Donsakul T (1993) A chromosome study on three Cyprinid fishes, *Labeo erythrus*, *L. bicolor* and *L. rohita*. *Proceedings of 31st Kasetsart University Annual Conference: Animal, Fisheries and Veterinary Medicine*. Kasetsart University, Bangkok, 543–551. [In Thai]

- Maneechot N, Yano CF, Bertollo LAC, Getlekha N, Molina WF, Ditcharoen S, Tengjaroenkul B, Supiwong W, Tanomtong A, Cioffi MB (2016) Genomic organization of repetitive DNAs highlights chromosomal evolution in the genus *Clarias* (Clariidae, Siluriformes). *Molecular Cytogenetics* (2016) 9: 1–4. <https://doi.org/10.1186/s13039-016-0215-2>
- Mantovani M, Abel LDS, Mestriner CA, Moreira-Filho O (2004) Evidence of the differentiated structural arrangement of constitutive heterochromatin between two populations of *Astyanax scabripinnis* (Pisces, Characidae). *Genetics and Molecular Biology* 27: 536–542. <https://doi.org/10.1590/S1415-47572004000400012>
- Martins C (2007) Chromosomes and Repetitive DNAs: A Contribution to the Knowledge of the Fish Genome. In: Pisano E, Ozouf-Costaz C, Foresti F (Eds) *Fish Cytogenetics*. Science Publishers, 421–453. <https://doi.org/10.1201/b10746-16>
- Mengampan K, Manosoy J, Meevetee A, Manosoy A (2004) Chromosomal Karyotyping from Peripheral Blood Lymphocytes of the Mekong Giant Catfish (*Pangasianodon gigas*, Chevey). *Thai Fisheries Gazette* 57(4): 349–351. [In Thai]
- Nagpure NS, Kumar R, Srivastava SK, Gopalakrishnan A, Verma MS, Baseer VS (2004) Cytogenetic studies of fish species *Horabagrus nigricollaris*, *Puntius denisonii* and *Puntius sarana subnasutus* endemic to the Western Ghats. *The Nucleus* 47: 143–148.
- Nagpure NS, Kushwaha B, Srivastava SK, Kumar R, Gopalakrishnan A, Baseer VS, Verma MS (2003) Characterization of three endemic fish species from Western Ghats using cytogenetic markers. *The Nucleus* 46: 110–114.
- Na-Nakorn U, Tarnchalanukit W, Taptippawan P (1980) Induction of triploidy in *Clarias batrachus* by cold shock. *Kasetsart Journal* 22: 116–121. [In Thai]
- Nelson JS (2006) *Fishes of the World* (4th ed.). John Wiley & Sons, New York.
- Nitikulworawong N, Khruanet W (2014) Karyotypic of golden little barb, *Puntius brevis* Bleeker, 1850 (Pisces: Cyprinidae) from Khon Kaen Province. *KKU Science Journal* 42(1): 106–118.
- Ohno S, Muramoto J, Christian L, Atkin NB (1967) Diploid-tetraploid relationship among Old-World members of the fish family Cyprinidae. *Chromosoma* 23: 1–9. <https://doi.org/10.1007/BF00293307>
- Ojima Y, Yamamoto K (1990) Cellular DNA contents of fishes determined by flow cytometry. *La Kromosomo, Series II* 57: 1871–1888.
- Pinkel D, Straume T, Gray J (1986) Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. *Proceedings of the National Academy of Sciences of the United States of America* 83: 2934–2938. <https://doi.org/10.1073/pnas.83.9.2934>
- Plohl M, Luchetti A, Meštrović N, Mantovani B (2008) Satellite DNAs between selfishness and functionality: structure, genomics and evolution of tandem repeats in centromeric (hetero) chromatin. *Gene* 409: 72–82. <https://doi.org/10.1016/j.gene.2007.11.013>
- Rishi KK (1973) Somatic karyotypes of three teleosts. *Genen Phaenen* 16(3): 101–107.
- Rishi KK (1981) Chromosomal studies on four cyprinid fishes. *International Journal of Academy of Ichthyology* 2: 1–4.
- Rishi KK, Rishi S (1981) Giemsa banding in fish chromosomes. *Perspectives in Cytology and Genetics* 3: 103–106.
- Rishi KK, Sharma MP, Mankotia R (1977) Somatic chromosomes of three Indian teleosts. *Matsya* 3: 6–9.

- Sahoo PK, Nanda P, Barat A (2007) Karyotypic analysis of *Neolissocheilus hexagonolepis* (McClelland), *Puntius ticto* (Ham.) and *P. chola* (Ham.) (family: Cyprinidae, Pisces). *Cytologia* 72: 409–413. <https://doi.org/10.1508/cytologia.72.409>
- Seetapan K (2007) Karyotypes of sex fish species of the family Cyprinidae. Proceedings of the 45th Kasetsart University Annual Conference: Fisheries. Kasetsart University, Bangkok, 749–758. [In Thai]
- Sermwatanakul A (2005) The Current State of Ornamental Fish Industry in Thailand. Extension Paper No. 2/2005. Ornamental Fish and Aquarium Plants Research Center, Department of Fisheries. [In Thai]
- Sharma OP, Agarwal A (1981) The somatic and meiotic chromosomes of *Puntius conchoniis* (Cyprinidae) from the Jammu and Kashmir state, India. *Genetica* 56: 235–237. <https://doi.org/10.1007/BF00057565>
- Sharma OP, Tripathi NK, Sharma KK (2002) A review of chromosome banding in fishes. In: Sobti RC (Ed.) *Some Aspects of Chromosome Structure and Functions*. New Narosa Publishing House, Delhi, 109–122. https://doi.org/10.1007/978-94-010-0334-6_11
- Sharma OP, Sharma KK, Tripathi NK, Gupta SC (1995) Localization of C-heterochromatin in two cyprinid fishes *Puntius ticto* and *Schizothorax niger*. *Chromosome Information Service* 58: 19–20.
- Sobita N, Bhagirath TH, Dhananjay CH (2004) Chromosome complements of a cyprinid fish *Puntius manipurensis*. *The Nucleus* 47: 59–63.
- Sofy HI, Layla AM, Iman MKA (2008) Karyotypic diversity of some tilapia species. *Nature and Science* 6(1): 19–27.
- Sophawanus M, Tanomthong A, Suphiwong W (2017) Standardized Karyotype and Idiogram of Sicklefin Barb (*Puntioplites falcifer*) in Thailand. *Koch Cha Sarn Journal of Science* 39(2): 75–84
- Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Pinthong K, Tanee T, Tanomthong A (2013) Karyotype and cytogenetic mapping of 9 classes of repetitive DNAs in the genome of the naked catfish *Mystus bocourti* (Siluriformes, Bagridae). *Molecular Cytogenetics* 6(1): 1–51. <https://doi.org/10.1186/1755-8166-6-51>
- Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Fan X, Tanee T, Tanomthong A (2014a) Comparative cytogenetic mapping of rRNA genes among naked catfishes: implications for genomic evolution in the Bagridae family. *Genetics and Molecular Research* 13(4): 9533–9542. <https://doi.org/10.4238/2014.November.12.2>
- Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Pinthong K, Tanee T, Tanomthong A (2014b) Chromosomal evolution in naked catfishes (Bagridae, Siluriformes): A comparative chromosome mapping study. *Zoologischer Anzeiger-A Journal of Comparative Zoology* 253(4): 316–320. <https://doi.org/10.1016/j.jcz.2014.02.004>
- Supiwong W, Tanomthong A, Jumruthanasan S, Khakhong S, Leelarasamee K, Sanoamuang L (2012) A first karyotype analysis and chromosomal characteristic of nucleolar organizer regions (NORs) of common sheathfish, *Micronema apogon* (Siluriformes, Siluridae) in Thailand. *Cytologia* 77(1): 53–58. <https://doi.org/10.1508/cytologia.77.53>
- Tanomthong A (2011) *Cytogenetics*. Khon Kaen University press, Khon Kaen. [in Thai]
- Vidthayanon C (2005) *Handbook of Freshwater Fish* (2nd ed.). Sarakadee Press, Bangkok, Thailand. [In Thai]