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First classical and molecular cytogenetic analyses of *Sperata acicularis* (Siluriformes, Bagridae)

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ABSTRACT: The first chromosomal analysis of Salween shovelhead catfish **(***Sperata acicularis***)** was undertaken by classical cytogenetic and fluorescence in situ hybridization (FISH) techniques in the present study. Ten male and ten female fish were obtained from Salween River, Mae Hong Son Province, Northern Thailand. The mitotic chromosome preparation was directly performed from kidney tissues**.** Conventional Giemsa staining, Ag-NOR staining, and molecular cytogenetics techniques with FISH using 5S, 18S rDNAs, and microsatellites d(CA)$_{15}$ and d(GC)$_{15}$ repeats as probes were conducted**.** The results indicated that the diploid chromosome number of *S. acicularis* was 2*n* = 56**.** The fundamental number **(**NF**)** was 110 both for males and females**.** The karyotype is composed of 18 large metacentric, 10 large submetacentric, 14 medium metacentric, 12 medium submetacentric and 2 medium acrocentric chromosomes; sex chromosomes could not be identified. NORs localized at the subtelomeric region of the short arm of metacentric chromosome pair 3, which coincides with location of 18S rDNA probe. 5S rDNA probe signal was detected on the short arm of the metacentric chromosome pairs 5 and 8. The distribution patterns of each analysed microsatellite repeat on the chromosomes differed from each other. Microsatellite d(CA)$_{15}$ repeats were highly accumulated at telomeric regions of all chromosome pairs, and throughout the chromosome in some pairs while the microsatellite d(GC)$_{15}$ repeats were scattered and less accumulated in some chromosome pairs. Overall, we present the karyotype of *S. acicularis* providing insights into species' evolution and enabling undoubtedly species identification.

KEY WORDS: Chromosome, FISH, karyotype, Nucleolar Organizer Regions, *Sperata acicularis.*

INTRODUCTION

Bagridae is a family of catfishes found in Africa and Asia, from Japan to Borneo, and consists of approximately 200 species (Eschmeyer, 2015). These species belong to the order Siluriformes and are commonly known as bagrid or naked catfishes (Ferraris, 2007). This family is the largest one of Thai catfishes. In Thailand, there are six genera including *Bagrichthys* Bleeker, 1857; *Batasio* Blyth, 1860; *Hemibagrus* Bleeker, 1862; *Mystus* Scopoli, 1777; *Pseudomystus* Jayaram, 1968 and *Sperata* Holly, 1939 (Vidthayanon, 2005). For the genus *Sperata*, there are currently four recognized species, namely Salween shovelhead catfish *S. acicularis*, long-whiskered catfish *S. aor*, *S. aorella* and giant river-catfish *S. seenghala*. Species of *Sperata* are found in Southern Asia from Afghanistan to Thailand, in a wide variety of water bodies. The *Sperata* species are sport angler fishes as well as being important food supplying fish (Froese and Pauly, 2011). Only one species, *S. acicularis* (Fig. 1A) is reported in Thailand that it is native fish to the Salween Basin. This species is booming in Thailand due to its role as fish food supplying species and ornamental fish (Vidthayanon, 2005). Although it has been recognized by science, there is still little information on its genome, and especially on its chromosomal content.

Up to the present, 45 species in family Bagridae have been cytogenetically analysed so far. Diploid chromosomes number varies between 2*n* = 44 (*Coreobagrus brevicorpus*) and 2*n* = 80 (*Batasio fluviatilis*); fundamental number (number of chromosome arms, NF) varies from 64 (*M. tengara* and *M. vittatus*) to 116 (*Horabagrus brachysoma* and *Ho. nigricollaris*) (Arai, 2011). However, only five cytogenetic studies of the genus *Sperata* (three from four species) have been reported (Table 1). The 2*n* ranged from 50 chromosomes

Table 1. Review of cytogenetic reports of the genus *Sperata* (family Bagridae).

Species	2*n*	NF	Karyotype formula	NORs	Reference
Sperata acicularis	56	100	30m+14sm+12a	–	Magtoon and Donsakul (2009)
	56	**110**	**32m+22sm+2a**	**2**	**Present study**
S. aor	52	96	20m+14sm+10st+8a	–	Lakra and Rishi (1991)
S. seenghala	54	102	28m+12sm+8st+6a	2	Rishi *et al*. (1994)
					Sharma and Tripathi (1986)
	50	80	10m+14sm+6st+20a	2	Das and Khuda-Bukhsh (2007a)

Notes*:* 2*n* = diploid chromosome number, NF = fundamental number *(*number of chromosome arm*)*, m = metacentric, sm = submetacentric, a = acrocentric, t = telocentric, NORs = nucleolar organizer regions, and – = not available*.*

in *S. seenghala* (Das and Khuda-Bukhsh, 2007a) to 56 chromosomes in *S. acicularis* (Magtoon and Donsakul, 2009). Chromosomal studies on fishes have been shown to be suited to provide new information about karyotypical variability at inter- and intra-specific levels, which can be of great interest to phylogenetics, systematics and taxonomy (Centofante *et al*., 2002). In addition, cytogenetic knowledge can be applied in breeding improvement (Na-Nakorn *et al*., 1980; Sofy *et al*., 2008) and cytotoxicology (Promsid *et al*., 2015; Supiwong *et al*., 2013b).

For some species, the karyotypic information from classical cytogenetics has been shown to be enough to identify variations among populations and species. However, in most cases, mere descriptions of karyotypes seem to be inconclusive when they are not used in combination with other methods to produce more accurate chromosome markers. In this sense, analyzes on the molecular cytogenetic level play an important role in precise characterization of the structure of fish genomes (Cioffi and Bertollo, 2012). Multiple DNA copies of 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 satellite DNAs; also there are dispersed elements, such as transposons and retrotransposons, known as transposable elements (TEs) (Jurka *et al*., 2005). Recently, molecular cytogenetic studies using fluorescence in situ hybridization (FISH) for mapping of repetitive DNA sequences have provided important contributions to the characterization of biodiversity and evolution of divergent fish groups (Cioffi and Bertollo, 2012). Moreover, some microsatellite repeats are species-specific characters among some fish groups (Cioffi *et al*., 2015). An important role of repetitive DNAs in genome evolution has been reported for different fish groups (Cioffi and Bertollo, 2012; Cioffi *et al*., 2010, 2015; Moraes *et al*., 2017, 2019; Sassi *et al*., 2019; Terencio *et al*., 2013; Yano *et al*., 2014). Nevertheless, there are only three studies in family Bagridae using FISH technique, as follows. The 5S and 18S rDNAs, U2 snRNA, the microsatellites $(CA)_{15}$ and $(GA)_{15}$, telomeric repeats, and the retrotransposable elements Rexs 1, 3 and 6 were analyzed in *M. bocourt* (Supiwong *et al*., 2013a). Additionally, the microsatellites $(CA)_{15}$ and $(GA)_{15}$, the retrotransposable element Rex 1, 18S, and 5S rDNAs as probes were hybridized on the chromosomes in nine species, including *Hemibagrus filamentus*, *He. spilopterus*, *He. wyckii*, *He. wyckioides*, *M. atrifasciatus*, *M. bocourti*, *M. multiradiatus*, *M. mysticetus* and *P. siamensis* (Supiwong *et al*., 2014a, b). Accordingly, FISH technique to investigate the chromosomal distribution of repetitive DNA sequences on the chromosomes of the genus *Sperata* fishes has not been performed.

Thus, the present study is the first report on chromosomal characteristics of NOR and FISH mapping of 5S and 18S rDNAs, the microsatellites $(CA)_{15}$, and $(GC)_{15}$ repeats in *S. acicularis* by using classical and molecular cytogenetic protocols**.** The knowledge gained can provide cytogenetic information potentially useful for further study on taxonomy and evolutionary relationship in this family.

MATERIALS AND METHODS

Sample collection

Ten males and ten females of *S. acicularis* were collected from the Salween River, Mae Hong Son Province, Northern Thailand. The fish were transferred to laboratory aquaria and kept under standard conditions for three days before the experiments. The procedures followed ethical protocols; anaesthesia was conducted by kept in freeze before euthanasia, as approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethics of Animal Experimentation of the National Research Council of Thailand ACUC-KKU-15/2559.

Classical cytogenetic study

Chromosomes were prepared *in vivo* (Supiwong *et al*. 2012, 2013b). Conventional staining technique was performed by using 20% Giemsa**'**s solution and Ag-NOR staining was conducted by using 50% silver nitrate solution (Howell and Black, 1980). Chromosome counting was performed on mitotic metaphase cells under a light microscope. Twenty cells (from each specimen), clearly observable and well-spread chromosomes were selected and photographed (selected from all specimens). The length of short arm (Ls) and long arm (Ll) were measured and the length of total chromosome was

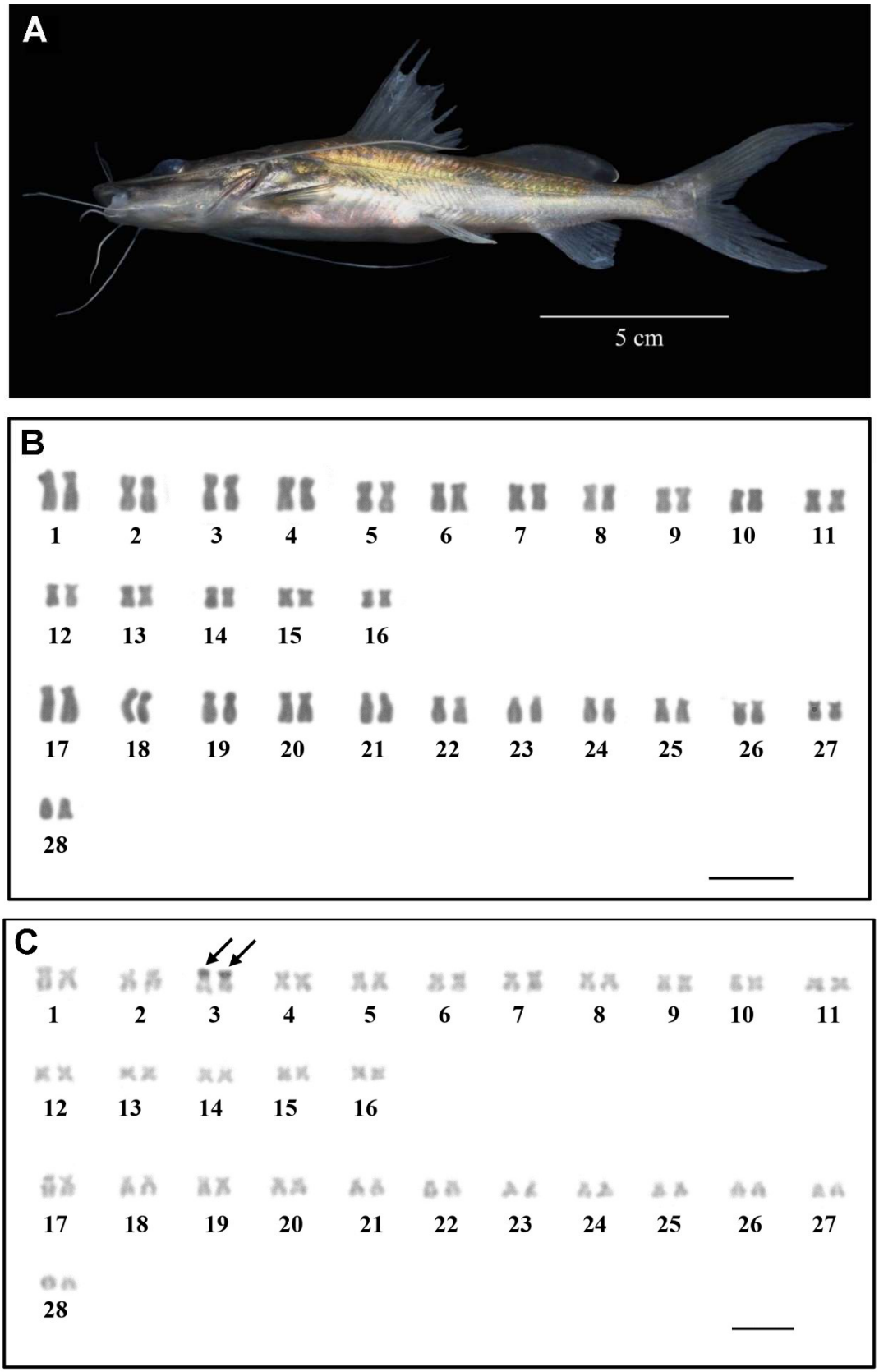

Fig. 1. ***Sperata acicularis*****,** 2*n* = 56. **A.** Specimen; karyotypes from **B.** conventional staining and **C.** Ag-NOR staining (arrows indicate Nucleolar Organizer Regions/NORs). Scale bars = 5 μm.

calculated (LT, LT = Ls+Ll). The relative length (RL), the centromeric index (CI) and standard deviation (S.D.) of RL and CI were calculated (Supiwong *et al*. 2013b). The CI (q/p) between 1.00–1.70, 1.70–3.00, 3.00–7.00, and 7.00–∞ were described as metacentric (m), submetacentric (sm), subtelocentric (st), and acrocentric (a) chromosomes, respectively (Levan *et al*., 1964).

Fluorescence in situ hybridization (FISH)

FISH was performed under high-stringency conditions on metaphase chromosome spreads with specific probes for 5S and 18S rDNAs. The 5S rDNA probe included the transcriptional segment of the 5S rRNA gene, with 120 base pairs (bp), and the 200-base pair non-transcribed spacer (NTS) (Martins *et al*. 2006). The 18S rDNA probe corresponding to a 1,400 base pair segment of the 18S rDNA gene (Cioffi *et al*., 2009). Both rDNA probes were directly labelled with the Nick-translation Labeling Kit (Jena Bioscience, Jena, Germany) by the fluorescent labels Atto488 (18S rDNA) and Atto550 (5S rDNA), according to the manufacturer's manual.

Microsatellites $d(CA)_{15}$, and $d(GC)_{15}$ probes (Kubat *et al*., 2008) were directly labelled with Cy3 at 5′ terminal during synthesis by Sigma (St. Louis, MO, USA). FISH under high stringency conditions on mitotic chromosome spreads (Pinkel *et al*., 1986) was carried out by previous protocals as reported by Supiwong *et al*. (2017a) and Yano *et al*. (2017). The hybridization signals were checked and analyzed on an epifluorescence microscope Olympus BX50 (Olympus Corporation, Ishikawa, Japan).

RESULTS

Chromosome number (2*n*), fundamental number (NF), and karyotype of *S. acicularis*

The diploid chromosome number (2*n*) of *S. acicularis* was 56 chromosomes. The fundamental number (NF) was 110 in both males and females (Fig. 1B). The karyotype comprised 18 large metacentric, 10 large submetacentric, 14 medium metacentric, 12 medium submetacentric, and 2 medium acrocentric chromosomes. The chromosomes length in micrometres from 20 metaphase cells (10 cells from males and 10 cells from females) in mitotic metaphase was measured. The mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), total length of arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation of RL, CI, size and type of chromosome are presented in Table 2. The heteromorphic sizes related to sex chromosomes were not identified. The karyotype formula for this species is as follows:

2*n* (diploid) 56 = L^{m}_{18}+L^{sm}_{10}+ M^{m}_{14}+M^{sm}_{12}+M^{a}_{2} or
2*n* (diploid) 56 = 32m+22sm+2a

Chromosome markers of *S. acicularis*

The determination of a chromosomal markers for this species was firstly obtained in the present study by using the Ag-NOR staining technique. The region adjacent to the telomeric region of the short arm of the metacentric chromosome pair 3 showed clearly observable NORs (Fig. 1C).

Patterns of 5S and 18S rDNAs and the microsatellite repeats on the genomes of *S. acicularis*

5S rDNA sequences were mapped to the short arms of the metacentric chromosome pairs 5 and 8. The 18S rDNA probe displayed coincident hybridization signals of NOR-bearing chromosomes, at the subtelomeric region of the short arms of the metacentric pair 3 (Figs. 2A, 3). The results of the mapping of microsatellite repeats on the chromosomes of *S. acicularis* showed that $d(CA)_{15}$ repeats are located on the telomeric regions in all chromosome pairs and throughout the chromosome of the pairs 5, 15, 16, 21, 22, and 24 (Fig. 2B). Controversially, the microsatellite $d(GC)_{15}$ repeats are scattered and less accumulated in some chromosome pairs (Fig. 2C).

DISCUSSION

Chromosome number (2*n*), fundamental number (NF), and karyotype of *S. acicularis*

S. acicularis had the 2*n* = 56 which is in accordance with one single previous report (Magtoon and Donsakul, 2009). However, it differs from other species in the genus that *S. aor* had 2*n* = 52 (Lakra and Rishi, 1991) whereas *S. seenghala* had 2*n* = 50 (Das and Khuda-Bukhsh, 2007a) or 2*n* = 54 (Rishi *et al*., 1994; Sharma and Tripathi, 1986). To compare with other species of distinct genera, the 2*n* of *S. acicularis* is same as that of *Coreobagrus ichikawai* (Ueno, 1985), *He. menoda* (Khuda-Bukhsh *et al*., 1995), *He. nemurus* (Khuda-Bukhsh *et al*., 1995; Sharma and Tripathi, 1986), *Mystus albolineatus* (Donsakul, 2000), *M. bocourti* (Donsakul, 2000; Supiwong *et al*., 2013a, 2014a, b), *M. bleekeri* (Chanda, 1989; Sharma and Tripathi, 1986), *M. ngasep* (Singh *et al*., 2013), *Pelteobagrus nudiceps* (Fujioka, 1973; Ojima *et al*., 1990; Ueno, 1985) and *Pseudobagus tokiensis* (Ueno, 1974). This 2*n* is considered as the ancestral diploid chromosome number of this family (Sharma and Tripathi, 1986), as well as for the whole order of Siluriformes (Oliveira and Gosztonyi, 2000). However, NF and karyotype in the present study were inconsistent with the previous study (Magtoon and Donsakul, 2009). The differences of NFs were caused by differences of karyotypes, which can either be regarded as the intra-specific variations among populations, and/or misidentification of species or different species due to complex species. Ghigliotti *et al*. (2007) proposed that species with a higher NF value are more advanced in evolutionary terms. The process involving chromosome evolution in this species may be an inversion, as differences of the number of bi-armed chromosomes have been seen here and in Magtoon and

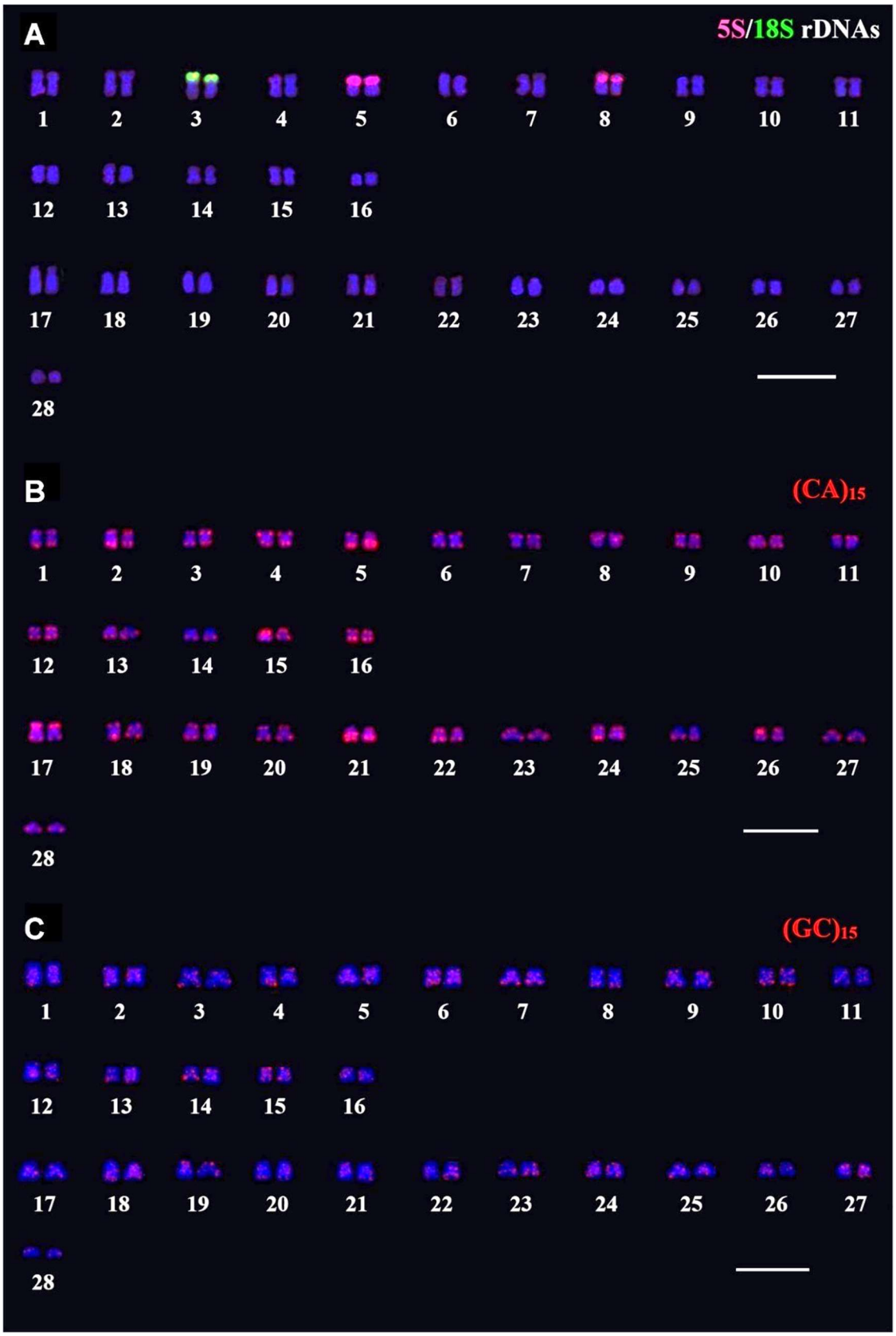

Fig. 2. Karyotypes of ***Sperata acicularis***, 2*n* **=** 56 by fluorescent *in situ* hybridization technique using **A.** 5S, 18S rDNAs; **B.** microsatellites $d(CA)_{15}$ and **C.** $d(GC)_{15}$ probes. scale bars = 5 µm.

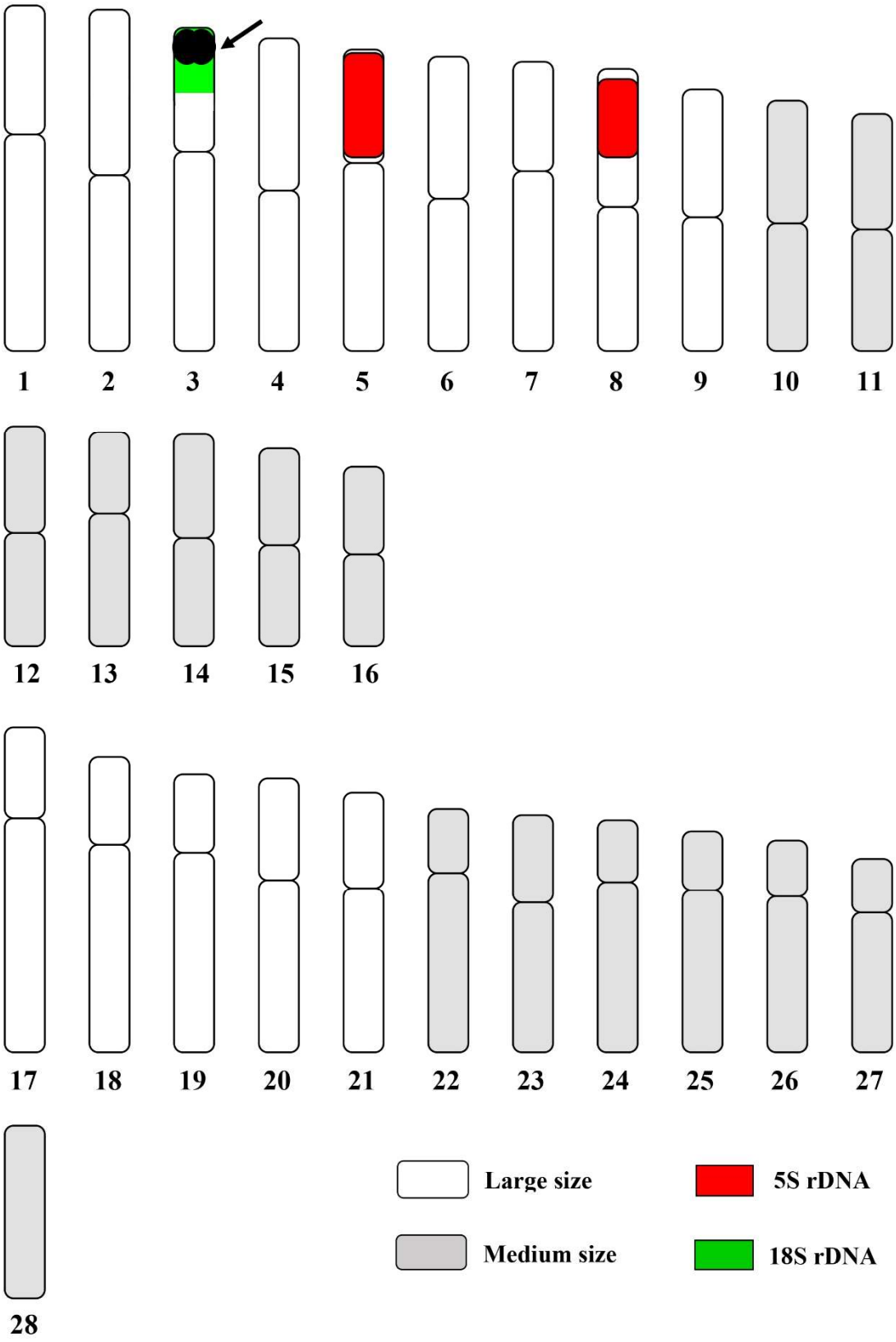

Fig. 3. Idiogram showing lengths and shapes of chromosomes of the ***Sperata acicularis***, 2*n* **=** 56, Color bars indicate rDNA probes after FISH technique (arrow indicates nucleolar organizer region/NOR)**.**

Table 2. Mean lengths of short arm chromosome (Ls), long arm chromosome (LI), total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL and CI from 20 metaphases of *Sperata acicularis*, (diploid) 2*n* = 56.

Chromosome pair	Ls	LI	LT	RL±SD	CI±SD	Chromosome type	Chromosome size
1	0.877	1.475	2.353	0.0478±0.0032	1.693±0.364	Metacentric	Large
2	1.126	1.200	2.326	0.0447±0.0034	1.061±0.044	Metacentric	Large
3*	0.840	1.360	2.201	0.0436±0.0032	1.632±0.218	Metacentric	Large
4	1.034	1.094	2.128	0.0418±0.0027	1.059±0.048	Metacentric	Large
5	0.775	1.282	2.056	0.0412±0.0023	1.676±0.208	Metacentric	Large
6	0.968	1.038	2.005	0.0402±0.0026	1.072±0.057	Metacentric	Large
7	0.743	1.227	1.969	0.0402±0.0026	1.654±0.200	Metacentric	Large
8	0.942	0.983	1.925	0.0394±0.0022	1.045±0.050	Metacentric	Large
9	0.871	0.915	1.786	0.0366±0.0022	1.053±0.053	Metacentric	Large
10	0.839	0.867	1.626	0.0333±0.0021	1.033±0.051	Metacentric	Medium
11	0.787	0.830	1.605	0.0330±0.0021	1.058±0.036	Metacentric	Medium
12	0.732	0.773	1.504	0.0308±0.0027	1.057±0.050	Metacentric	Medium
13	0.558	0.905	1.463	0.0296±0.0026	1.630±0.230	Metacentric	Medium
14	0.710	0.739	1.444	0.0296±0.0026	1.043±0.046	Metacentric	Medium
15	0.666	0.687	1.352	0.0277±0.0024	1.032±0.029	Metacentric	Medium
16	0.594	0.629	1.223	0.0249±0.0042	1.059±0.040	Metacentric	Medium
17	0.616	1.604	2.220	0.0441±0.0031	2.621±0.572	Submetacentric	Large
18	0.601	1.419	2.020	0.0430±0.0030	2.454±0.485	Submetacentric	Large
19	0.533	1.365	1.898	0.0393±0.0043	2.628±0.514	Submetacentric	Large
20	0.691	1.179	1.870	0.0381±0.0027	1.719±0.180	Submetacentric	Large
21	0.654	1.119	1.773	0.0362±0.0024	1.723±0.174	Submetacentric	Large
22	0.437	1.227	1.664	0.0340±0.0025	2.984±0.671	Submetacentric	Medium
23	0.598	1.027	1.625	0.0330±0.0022	1.751±0.229	Submetacentric	Medium
24	0.425	1.161	1.586	0.0324±0.0024	2.976±0.854	Submetacentric	Medium
25	0.407	1.106	1.513	0.0308±0.0025	2.816±0.733	Submetacentric	Medium
26	0.384	1.063	1.446	0.0295±0.0023	2.879±0.538	Submetacentric	Medium
27	0.365	0.956	1.322	0.0270±0.0025	2.808±0.676	Submetacentric	Medium
28	0.000	1.177	1.177	0.0243±0.0036	∞±0.000	Acrocentric	Medium

Note: * = NOR-bearing chromosomes.

Donsakul (2009). The NF in this genus may vary from 80 to 110 (Table 1). Although this species seems to be conserved for 2*n*, the karyotype is considered as advance state due to having a few mono armed chromosomes in karyotype composition and a great NF value. Similar to most species analyzed in the Bagridae family, no cytologically heteromorphic sex chromosome was observed in *S. acicularis*. Sex chromosome system in this fish might be related to the use of different master sex-determining genes, and the quest for such genes in different fish species might uncover currently unknown vertebrate sex-determining genes. Moreover, heteromorphic sex chromosomes in this fish and family might be initial step of differentiation. Thus, it cannot be detected by using cytogenetic study level. Nevertheless, there are only two species, such as *M. gulio* and *M. tengara*, which have differentiated sex chromosome systems as XX/XY and ZZ/ZW, respectively (Arai, 2011). Thus, the Bagridae family is in the early stages of the differentiation of sex chromosomes.

Chromosome markers of *S. acicularis*

Mapping of nucleolar organizer regions (NORs) in this species was performed here for the first time. If these regions are active during the interphase before mitosis, they can be detected by silver nitrate staining (Howell and Black, 1980) since they specifically stain a set of acidic proteins related to the ribosomal synthesis process; this technique reveals active NORs but is not specific to the inactive rDNA associated to NORs (Jordan, 1987).

The single NOR-bearing chromosome pair in the present study is in consistency with *S. seenghala* (Das and Khuda-Bukhsh, 2007a; Sharma and Tripathi, 1986) and only 2 NORs per genome is a common characteristic found in many species of this family such as *Bagrichthys majusculus* (Supiwong *et al*., 2018), *He. menoda* (Barat and Khuda-Bukhsh, 1986), *He. wyckii* (Supiwong *et al*., 2017b), *Horabagrus brachysoma* (Nagpure *et al*., 2003), *Ho. nigricollaris* (Nagpure *et al*., 2004), *M. cavasius* (Rishi *et al*., 1994; Sharma and Tripathi, 1986), *M. gulio* (Das and Khuda-Bukhsh, 2007b), *M. vittatus* (John *et al*., 1992; Khuda-Bukhsh and Barat, 1987), *Pelteobagrus ussuriensis* (Kim *et al*., 1982), *Pseudobagus vachelii* (Ueno, 1985), *Pseudomystus siamensis* (Supiwong *et al*., 2013b), and *Rita rita* (Khuda-Bukhsh and Barat, 1987). However, species *Tachysurus fulvidraco* had two NOR pairs (Zhang *et al*., 1992). A single NOR pair is considered as a primitive state in fish group. Thus, this

genus as well as this family have the conserved NOR number as the ancestral character of the catfish. This character is quite different from silurid catfish (Ditcharoen *et al*., 2019). In fishes, the location of NORs in a terminal position is also considered as a primitive characteristic (Vitturi *et al*., 1995). The locations of NORs of *S. acicularis* in the present study are the regions adjacent to the telomere of the short arm of the metacentric chromosome pair 3. Thus, this fish seems to be part of primitive trait due to its NOR position.

Patterns of 5S and 18S rDNAs and the microsatellite repeats on the genomes of *S. acicularis*

The mapping of the 18S rDNA sequences was shown on a single chromosome pair in *S. acicularis*, chromosome pair 3, which is coincident to NOR positions. This result is the same as seen in other nine species of the Bagridae family (Supiwong *et al*., 2014b): *He. filamentus*, *He. spilopterus*, *He. wyckioides*, *M. atrifasciatus*, *M. bocourti*, *M. multiradiatus*, *M. mysticetus*, *He. wyckii* and *P. siamensis*. However, in last two species interstitial 18S rDNA sites where on the long arm of meta-submetacentric chromosomes (Supiwong *et al*., 2014b). From previous and present studies, the 18S rDNA clusters were mostly located at the terminal region in Bagrids, except for *He. wyckii* and *P. siamensis*, representatives belonging to different genera. This species and the genus *Mystus* share the most conservative patterns concerning number and position of 18S rDNA clusters. Moreover, a single pair of 18S rDNA loci was identified in several fish species, namely *Channa lucius* (Cioffi *et al*., 2015), *Abudefduf* genus (Getlekha *et al*., 2016a), *Dascyllus* genus (Getlekha *et al*., 2016b), *Clarias gariepinus* and *C. macrocephalus* (Maneechot *et al*., 2016), *Belodontichthys truncatus*, *Kryptopterus limpok*, *K. macrocephalus*, *Micronema cheveyi*, *Ompok fumidus*, *Phalacronotus apogon*, *P. bleekeri*, and *Wallago attu* (Ditcharoen *et al*., 2019).

In contrast, the 5S rDNA probe was localized at the short arm of metacentric chromosome pairs 5 and 8. The number of 5S rDNA sequences in *S. acicularis* is the same as in *He. spilopterus* (Supiwong *et al*., 2014b). In addition, this distribution is the same as for other catfishes including *Clarias gariepinus* (Maneechot *et al*., 2016) and *Kryptopterus geminus* (Ditcharoen *et al*., 2019). However, it is different from Bagrids such as *He. wyckii* (synteny between the 18S and 5S rDNA sites), *He. wyckioides* (one pair of 5S rDNA), *M. atrifasciatus* (one pair of 5S rDNA), *M. bocourti* (one pair of 5S rDNA), *M. multiradiatus* (one pair of 5S rDNA), *M. mysticetus* (one pair of 5S rDNA), *P. siamensis* (one pair of 5S rDNA), *He. wyckii* (three pairs of 5S rDNA) and *He. filamentus* (five pairs of 5S rDNA) (Supiwong *et al*., 2014b). Multiple loci of minor ribosomal genes have also been found in some catfishes (Ditcharoen *et al*., 2019; Maneechot *et al*., 2016) and a great number of 5S rDNA loci was also observed in *C. batrachus* with 54 chromosomes harboring 5S rDNA positions (Maneechot *et al*., 2016).

Ribosomal DNAs can spread through the genome, thus creating new rDNA loci, variant rDNA copies and even associations with other multigene families and transposable elements (Martins *et al*., 2006). The existence of multiple 5S rDNA loci in many chromosome pairs could mean that these clusters have been evolving independently and are represented by different units of 5S rDNA (Martins *et al*., 2002). Thus, the existence of multiple minor ribosomal gene loci appears to represent a derived condition in the evolutionary dynamics of fishes (Martins and Galetti, 1999). Up to the present, the genera *Mystus*, *Pseudomystus* and *He. wyckioides,* share the plesiomorphic condition (primitive stage) for this character, while in *He. nemurus, He. wyckii He. filamentus* and *S. acicularis* could have undergone some mechanism such as non-homologous crossing-over to give rise to the derived condition of multiple 5S rDNA loci. The extensive variation in number and chromosomal position of rDNA clusters that was observed among Bagridae fishes seems to be related to the intense evolutionary dynamics of repeated units of rDNA that generates divergent patterns of chromosomal distribution even among closely related species (Supiwong *et al*., 2014b).

Chromosomal mapping of microsatellite $(CA)_{15}$ and $(GC)_{15}$ repeats in *S. acicularis* was also done. Microsatellite $(CA)_{15}$ repeats were abundantly located distributed in all chromosomes, usually in telomeric regions, and few chromosome pairs showed the strongest signal intensities. The present result is comparable to that as observed in *M. bocourti*. This could mean that *M. bocourti* and *S. acicularis* have the accumulation of a species-specific type of heterochromatin (Supiwong *et al*. 2013a, 2014a). Moreover, the patterns of microsatellite $(CA)_{15}$ repeats in this species are similar to other Bagrid catfishes including *He. filamentus*, *He. spilopterus*, *He. wyckii He. wyckioides*, *M. atrifasciatus*, *M. multiradiatus*, *M. mysticetus*, *P. siamensis* (Supiwong *et al*., 2014a), *Clarias* species (Maneechot *et al*., 2016), *Catlocarpio siamensis* and *Probarbus jullieni* (Saenjundaeng *et al*., 2018). In turn, the microsatellite $(CA)_{15}$ sequences displayed a scattered distribution throughout most of the chromosomes, without preferential accumulation. In *Dascyllus* species and in *D. aruanus*, they were found in the short arms of the pair no. 10, co-located with the 18S rDNA position (Getlekha *et al*., 2016b). For the mapping of $(GC)_{15}$ repeats in *S. acicularis*, they were distributed among all chromosomes, not really being suited to serve as chromosomal markers. However, the pattern of microsatellite $(GC)_{15}$ is different in *Channa gachua*, *C. lucius*, *C. micropeltes*, *C. striata* (Cioffi *et al*., 2015), *Toxotes chatareus* (Supiwong *et al*., 2017a), and Asian swamp eel, *Monopterus albus* (Supiwong *et al*., 2019).

Overall, it is believed that the microsatellites have specific zones of accumulation in genomes, preferentially in heterochromatic regions (Supiwong *et al*., 2014a). In fact, microsatellites are located in the heterochromatic regions (telomeres, centromeres, and in the sex chromosomes) of fish genomes (Cioffi and Bertollo, 2012), including the present study. However, the distribution of microsatellites was not only restricted to heterochromatin, but also dispersed in euchromatic regions of the chromosomes (Getlekha *et al*., 2016b). Nonetheless, closely related fish species involved in recent speciation events could present a differential pattern in the distribution and quantity of microsatellite sequences on chromosomes.

In conclusion, we present the first molecular cytogenetic karyotype of *S. acicularis*, providing insights into species' evolution and enabling undoubtedly species identification.

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