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# Evolutionary tracks of chromosomal diversification in *Trichopsis* (Anabantiformes, Osphronemidae) fishes: New insights from a molecular cytogenetic perspective

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**Abstract.** *Mingkwan B, Sassi FDMC, Muanglenm N, Pinmongkhonkul S, Pinthong K, Tongnunui S, Yeesin P, Tanomtong A, Liehr T, Cioffi MDB, Supiwong W. 2023. Evolutionary tracks of chromosomal diversification in* Trichopsis *(Anabantiformes, Osphronemidae) fishes: New insights from a molecular cytogenetic perspective. Biodiversitas 24: 1551-1559.* This study investigated the chromosomes of three *Trichopsis* species (namely *Trichopsis pumila*, *Trichopsis vittata*, and *Trichopsis schalleri*) using conventional (Giemsa stain, Ag-NOR) and molecular cytogenetic techniques. Fluorescence in situ hybridization )FISH( with repetitive DNAs, including 5S and 18S rDNAs, and microsatellites as probes were also performed. Our results indicated a conserved diploid number of 46 (2*n*) for all analyzed species of both sexes, although varying in their karyotype structure. Furthermore, *T. schalleri* and *T. vittata* karyotypes were presented with only acrocentric chromosomes (46a), while *T. pumila* had a submetacentric pair in addition to acrocentric ones (2sm+44a). Positive Ag-NOR sites were differentially found in each species adjacent to the acrocentric chromosome's centromeric and/or interstitial sub-centromeric regions. Both 5S and 18S rDNAs vary between the analyzed species, but all were localized in multiple sites. However, the distribution pattern of microsatellites differed in each species, mostly scattering at peri-centromeric and telomeric regions. These findings show that *Trichopsis* species, while having a conserved diploid number, differ significantly in the distribution of repetitive sequences in their karyotypes, particularly for rDNAs. These species-specific patterns can be useful for characterizing and identifying further different species and examining the evolutionary mechanisms that drove the evolution of these fishes' karyotypes.

**Keywords**: Chromosome, gouramies, microsatellites, nucleolar organizer regions, rDNAs

## INTRODUCTION

The fishes from the Anabantoidei (Anabantiformes) are known as "labyrinth fishes" because of an air-breathing accessory apparatus )super branchial organ( in the head region "called labyrinth organ" that serves as a lung and allows them to use ambient oxygen (Nelson et al. 2016). In addition to their eye-catching patterns, this characteristic makes several species in the three well-known families Anabantidae, Helostomidae, and Osphronemidae (Nelson et al. 2016; Taki et al. 2021) suitable for aquariums. However, the *Trichopsis* genus (Osphronemidae), well known by its common name "gouramies," includes only three valid species, all frequently observed in trade: the croaking gourami *Trichopsis vittata* Cuvier, 1831, the pygmy gourami *Trichopsis pumila* Arnold, 1936, and the three stripe gourami *Trichopsis schalleri* Ladiges, 1962. They resemble *Trichopodus* fishes in some ways; however, they are smaller and have a lance-shaped caudal fin (Taki et al. 2021). Gouramies can be found in a variety of water habitats, including marshes, ponds, reservoirs, irrigation canals, and slow-flowing rivers with dense aquatic vegetation, particularly in the Mekong basin in Laos, Thailand, Cambodia, and Vietnam, as well as the Chao Phraya basin and Sundaland (Taki et al. 2021).

Chromosomal studies in fishes have provided insights into the evolution: at inter-and intra-specific levels, phylogenetics, systematics, taxonomy, and genetic diversity (Kumar et al. 2014; Maneechot et al. 2016; Ditcharoen et al. 2019; Ditcharoen et al. 2020; Chaiyasan et al. 2021). The repetitive DNA fraction of several fish species have been used to explain the evolution and relationships of various fish families (Esmaeili et al. 2015; Getlekha et al. 2016a; Maneechot et al. 2016; Ditcharoen et

al. 2019; Ditcharoen et al. 2020; Chaiyasan et al. 2021). For some fish species, however, challenges include obtaining enough live samples for chromosome analysis and increasing the quantity and quality of metaphase spreads, especially in populations with tiny chromosomes (Sember et al. 2020). According to data obtained in Table 1, the karyotypes of fishes belonging to the Osphronemidae family showed a remarkable diversity of diploid numbers (2*n*). These diploids range from 2*n*=16 in *Sphaerichthys osphromenoides* (Arai 2011) to 2*n*=48 in *Colisa chuna, Colisa fasciata, Helostoma temminckii, Trichogaster chuna, Trichogaster fasciata, Trichogaster labiosa,* and *Trichogaster sumatranus* (Arai 2011; Mingkwan et al. 2021). At the same time, Fundamental Number )NF( chromosome represented distinctiveness as 20-86 in *Luciocephalus pulcher* and *Trichogaster fasciata*, respectively (Arai 2011(.

Therefore, the chromosomal studies of *Trichopsis* are still developing. The cytogenetic studies on Osphronemidae are restricted to conventional cytogenetics protocols. Here**,** we aim to define the general information of fish chromosomes and provide the distribution of repetitive DNA sequences of *Trichopsis* in Thailand. The karyotype construction and localization of NORs were elucidated using conventional staining and Ag-NOR banding, respectively. Whereas 5S rDNA and 18S rDNA probes, microsatellite probes as d)CA($_{15}$, d)GC($_{15}$, d)CAG($_{10}$, d)CAC($_{10,}$ and d)GAA($_{10}$ were applied to determine, the repetitive DNA repeats on chromosomes among *Trichopsis* species. Thereby, the information from the present study may not only help to explain the comparative framework but also provide new insights into the chromosomal organization, taxonomy, and systematics.

## MATERIALS AND METHODS

### Sample preparation

The six males and six females of each analyzed species, *T. pumila, T. schalleri,* and *T. vittata,* were collected from two river basins in Thailand. Then, following *T. pumila* and *T. vittata* in Kwai Basin, Kanchanaburi Province (14°06'47.8"N 99°08'41.7" E), and *T. schalleri* in Chi Basin, Maha Sarakham Province (16°19'10.1"N 102°59'25.4" E). The alive fishes were transferred to the laboratory and kept in a well-aerated aquarium at 20-25°C before analysis. Then, the taxonomic identification was conducted following Kottelat (2001), Nelson et al. )2016(, Arai )2011(, and Taki et al. )2021(. Chromosomal preparation was directly performed in vivo. All specimens were injected intraperitoneally with 1 mL/100 g of body weight of 0.5% colchicine and sacrificed after one hour. (Kumar et al. 2013; Kumar et al. 2014; Khakhong et al. 2014; Kasiroek et al. 2017; Chaiyasan et al. 2018; Mingkwan et al. 2021(. The procedures of ethical protocols were performed under the approval of the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethics of Animal Experimental of the National Research Council of Thailand ACUC-KKU-39/2563.

### Conventional cytogenetics

Conventional staining and Ag-NOR banding were done using 20% Giemsa's solution and 50% silver nitrate solution adapted from Sangpakdee et al. )2015(, Sangpakdee et al. )2017), Jantarat et al. (2017), Pinthong et al. (2017), Sreeputhorn et al. )2017(, Supiwong et al. )2017(, Getlekha and Tanomtong )2020). With a Cannon D-150 camera, 20 metaphase spreads were recorded for each sample. The chromosomal characterization was performed using Microsoft Excel 2013 software and Adobe Photoshop CS6.5.2.3. The parameters of chromosomal analysis, including length of the short arm )Ls(, length of the long arm )Ll(, length of total chromosome )LT(, relative length )RL(, centromeric index )CI(, and standard deviation, were calculated. The chromosomal classification was further determined by Levan et al. )1964(.

### Molecular cytogenetics

The FISH technique was performed under high-stringency conditions using rDNA and microsatellite probes. The 5S rDNA probe was generated according to Pendas et al. (1994) and comprised 120 base pairs )bp( of the 5S rDNA gene and 200 bp of the non-transcribed spacer, while the 18S rDNA probe was generated following Cioffi et al. (2009) and was composed of 1,400 bp of 18S rDNA gene. The 5S and 18S rDNA probes were directly labeled with SpectrumOrange-dUTP and SpectrumGreen dUTP using nick translation, according to the manufacturer's recommendations )Roche, Mannheim, Germany(. The microsatellite probes d)CA($_{15}$, d)GC($_{15}$, d)CAG($_{10}$, d)CAC($_{10,}$ and d)GAA($_{10}$ were labeled with Cy3 at the 5' terminal during synthesis by Sigma )St. Louis, MO, USA(. The procedures were carried out following previous investigations in related groups (Getlekha et al. 2016a; Maneechot et al. 2016; Ditcharoen et al. 2019; Ditcharoen et al. 2020; Chaiyasan et al. 2021( with slight modifications. Then, the fluorescence signals were checked and analyzed by an epifluorescence microscope Olympus BX50 )Olympus Corporation, Ishikawa, Japan(.

## RESULTS AND DISCUSSION

### Diploid chromosome number *(*2*n),* Fundamental Number *(*NF*),* and karyotype of *Trichopsis*

All *Trichopsis* species presented 2*n*=46, *T. schalleri* and *T. vittata* showed 23 pairs of acrocentric chromosomes with NF=46. On the other hand, *T. pumila* presented one pair of submetacentric and 22 pairs of acrocentric chromosomes with the NF=48. The standardized karyotypes of *Trichopsis* were measured and revealed as one pair of large submetacentric, including: nine pairs of large and 13 pairs of medium acrocentric chromosomes in *T. pumila* (**L**$^{sm}_{2}$+L$^{a}_{18}$+M$^{a}_{26}$), six pairs of large, 17 pairs of medium acrocentric chromosomes in *T. schalleri* (L$^{a}_{12}$+S$^{a}_{34}$), and five pairs of large, 17 pairs of medium and one pair of small acrocentric chromosomes in *T. vittata* (L$^{a}_{10}$+M$^{a}_{34}$+S$^{a}_{2}$). Positive Ag-NOR sites were found pericentromeric in chromosome pairs 4 and 5 for *T. pumila* and sub-centromeric in pairs 2 and 3 for *T. schalleri,* while *T. vittata* only presented in pair 9 )Figure 1(.

**Table 1.** Review of cytogenetic reports of the family Osphornemidae

| Species | 2*n* | NF | Karyotype formula | NORs | Reference |
|---|---|---|---|---|---|
| *Colisa chuna* | 48 | 68 | 20m+12st+16a | - | Arai )2011( |
| *Colisa fasciata* | 48 | 80 | 16m+16sm+16a | - | Arai )2011( |
| *Colisa lalia* | 46 | 60 | 24m+22a | - | Arai )2011( |
| *Colisa labiosa* | 48 | 68 | 20m+10st+18a | - | Arai )2011( |
| *Helostoma temminckii* | 48 | 48 | 48t | 2 | Mingkwan et al. )2021( |
| *Luciocephalus pulcher* | 20 | 20 | 20a | - | Arai )2011( |
| *Sphaerichthys osphromenoides* | 16 | 30 | 10m+4sm+2a | - | Arai )2011( |
| *Trichogaster chuna* | 48 | 78 | 18m+12sm+18a/t | - | Arai )2011( |
| *Trichogaster fasciata* | 48 | 83 | 15m+16sm+4st+13a/t | 6 | Arai )2011( |
| | 48 | 86 | 16m+16sm+6st+10a/t | 2 | Arai )2011( |
| *Trichogaster labiosa* | 48 | 68 | 20m+10st+18a/t | - | Arai )2011( |
| *Trichogaster lalius* | 46 | 66 | 20m+8st+18a/t | - | Arai )2011( |
| *Trichogaster sumatranus* | 48 | 48 | 48st/a | - | Arai )2011( |
| *Trichopodus cantoris* | 46 | 46 | 46a/t | - | Arai )2011( |
| *Trichopodus leeri* | 46 | 46 | 46a/t | - | Abu-Almaaty et al. )2017( |
| | 46 | 46 | 46t | 2 | Supiwong et al. )2021( |
| *Trichopodus microlepis* | 46 | 46 | 46a/t | - | Arai )2011( |
| | 46 | 46 | 46t | 2 | Supiwong et al. )2021( |
| *Trichopodus pectoralis* | 46 | 46 | 46a/t | - | Arai )2011( |
| | 46 | 46 | 46t | 2 | Supiwong et al. )2021( |
| *Trichopodus trichopterus* | 46 | 46 | 46t/t | 2 | Arai )2011( |
| | 46 | 46 | 46a/t | - | Abu-Almaaty et al. )2017( |
| | 46 | 46 | 46t | 2 | Supiwong et al. )2021( |
| *Trichopodus cantoris* | 46 | 46 | 46a | - | Arai )2011( |
| *Trichopodus microlepis* | 46 | 46 | 46a | | Arai )2011( |
| *Trichopodus pectoralis* | 46 | 46 | 46a | | Arai )2011( |
| *Trichopodus leeri* | 46 | 46 | 46a | - | Arai )2011( |
| *Trichopodus sumatranus* | 46 | 46 | 46st/a | | Arai )2011( |
| *Trichopodus trichopterus* | 46 | 46 | 46a | | Arai )2011( |
| | 46 | 46 | 46a | 2 | Arai )2011( |
| *Trichopsis pumila* | 46 | 48 | 2m+44a | - | Donsakul et al. )2009( |
| | 46 | 48 | 2sm+44a | 4 | Present study |
| *Trichopsis schalleri* | 46 | 46 | 46a | - | Donsakul et al. )2009( |
| | 46 | 46 | 46a | 4 | Present study |
| *Trichopsis vittata* | 46 | 46 | 46a | - | Magtoon et al. )2007( |
| | 46 | 46 | 46a | 2 | Present study |
| *Beta imbellis* | 42 | 64 | 14m+8sm+4st+16a | - | Arai )2011( |
| *Beta prima* | 34 | 42 | 4m+4sm+4st+22a | - | Arai )2011( |
| *Beta simplex* | 44 | 52 | 4m+4sm+36a | - | Arai )2011( |
| *Beta smaragdina* | 42 | 48 | 2m+4sm+36a | - | Arai )2011( |
| *Beta splendens* | 42 | 54 | 4m+8sm+30a | - | Arai )2011( |
| *Belontia hasselti* | 48 | 48-49 | 48t )M(, 47t +1m)F( | 2 | Chaiyasan et al. )2021( |
| *Macropodus chinensis* | 48 | 56 | 6m+2sm+40a | - | Arai )2011( |
| *Macropodus concolor* | 46 | 64 | 8m+8sm+14st+16a | - | Arai )2011( |
| *Macropodus ocellatus* | 46 | 62 | 8m+8sm+14st+16a | - | Arai )2011( |
| *Macropodus opercularis* | 46 | 58 | 12m/sm+34st/a | - | Arai )2011( |
| *Macropodus spechti* | 46 | 58 | 10m+2sm+22st+12a | - | Arai )2011( |
| *Osphronemus exodon* | 48 | 48 | 48a | - | Arai )2011( |
| *Osphronemus goramy* | 48 | 48 | 48a | - | Arai )2011( |
| *Osphronemus laticlavius* | 48 | 50 | 2sm+46a | - | Arai )2011( |
| *Parosphromenus sumatranus* | 46 | 46 | 46a | - | Arai )2011( |

Note: 2*n*: diploid chromosome number. FN: Fundamental Number )number of chromosome arm(. m: metacentric, sm: submetacentric, a: acrocentric, t: telocentric, NORs: nucleolar organizer regions, and -: not available

**Patterns of repetitive DNA sequences on chromosomes of *Trichopsis***

Both 5S and 18S rDNA sequences have species-specific patterns in *Trichopsis*. In addition, *T. schalleri* and *T. pumila* karyotypes have seven (pairs 1, 2, 3, 14, 15, 16, and 20) and six (pairs 2, 4, 5, 6, 12, and 17) pairs, respectively. Those revealed 5S rDNA sites in pericentromeric regions. On the other hand, only four chromosomes (pairs 1, 3, 7, and 9) were found carrying these sequences in *T. vittata*. The 18S rDNA position corresponds to the Ag-NOR sites. Its followed by pericentromeric chromosome pair 9 in *T. Vittata*. While the interstitial site is closer to the centromeric region of chromosome pairs 4, 5, and 14 in *T. Pumila*, and of chromosome pairs 2 and 3 in *T. schalleri*

)Figure 2). Microsatellites also presented a variable pattern across the studied species. While $(GC)_{15}$ displayed a dispersion pattern for *T. pumila* and *T. vittata,* collected in pericentromeric areas in *T. schalleri.* In contrast, the $(CA)_{15}$ motif was accumulated in the pericentromeric region of nearly some chromosomes in *T. vittata* and *T. schalleri.* Therefore, it represented throughout the chromosome in *T. pumila,* and *T. schalleri*, the $(CAC)_{10}$ had an accumulation in one chromosomal pair, referring to NOR sites. In contrast, *T. pumila* and *T. vittata* displayed sporadic signals in several chromosomes' pericentromeric and telomeric regions. While the $(GAA)_{10}$ was differently mapped at centromeric and telomeric regions of the majority of chromosomes in each species. All *Trichopsis* showed signals of $(CAG)_{10}$ in the telomeric position (Figure 3).

## Discussions

All *Trichopsis* karyotypes demonstrated the same 2*n*=46, including 23 pairs of acrocentric chromosomes in *T. schalleri* and *T. vittata*, one submetacentric, and 22 pairs of acrocentric chromosomes in *T. pumila.* While *T. pumila* disclosed a different NF of 48, *T. schalleri* and *T. vittata* revealed their NFs to be 46, as already reported in the literature (Magtoon et al. 2007; Donsakul et al. 2009). Previous investigations with *T. pumila* chromosomes (Donsakul et al. 2009) revealed 23 acrocentric pairs with 2*n*=46 and NF=46, which falls into the known range of diploid and fundamental numbers of other Osphronemidae species **(**Magtoon et al. 2007; Donsakul et al. 2009; Arai 2011; Abu-Almaaty et al. 2017; Mingkwan et al. 2021; Supiwong et al. 2021(.

Moreover, due to that, a higher NF was referred to be the apomorphic character for Osphronemidae species and the karyotype with 2*n*=48, which was considered as the plesiomorphic karyotype of this family )Arai 2011; Gornung 2013; Nelson et al. 2016; Sochorová et al. 2018; Mingkwan et al. 2021; Supiwong et al. 2021(. *T. pumila* has more apomorphic cytogenetic characters than *T. schalleri* and *T. vittata*. The hypothesis of karyotype differentiation has been referred to as the association of intra-specific variation among the population. While chromosomal rearrangement as deletions, pericentric and paracentric inversions, Robertsonian rearrangement, and chromosomal translocation )Esmaeili et al. 2015; Getlekha et al. 2016a; Maneechot et al. 2016; Ditcharoen et al. 2019; Ditcharoen et al. 2020; Chaiyasan et al. 2021).

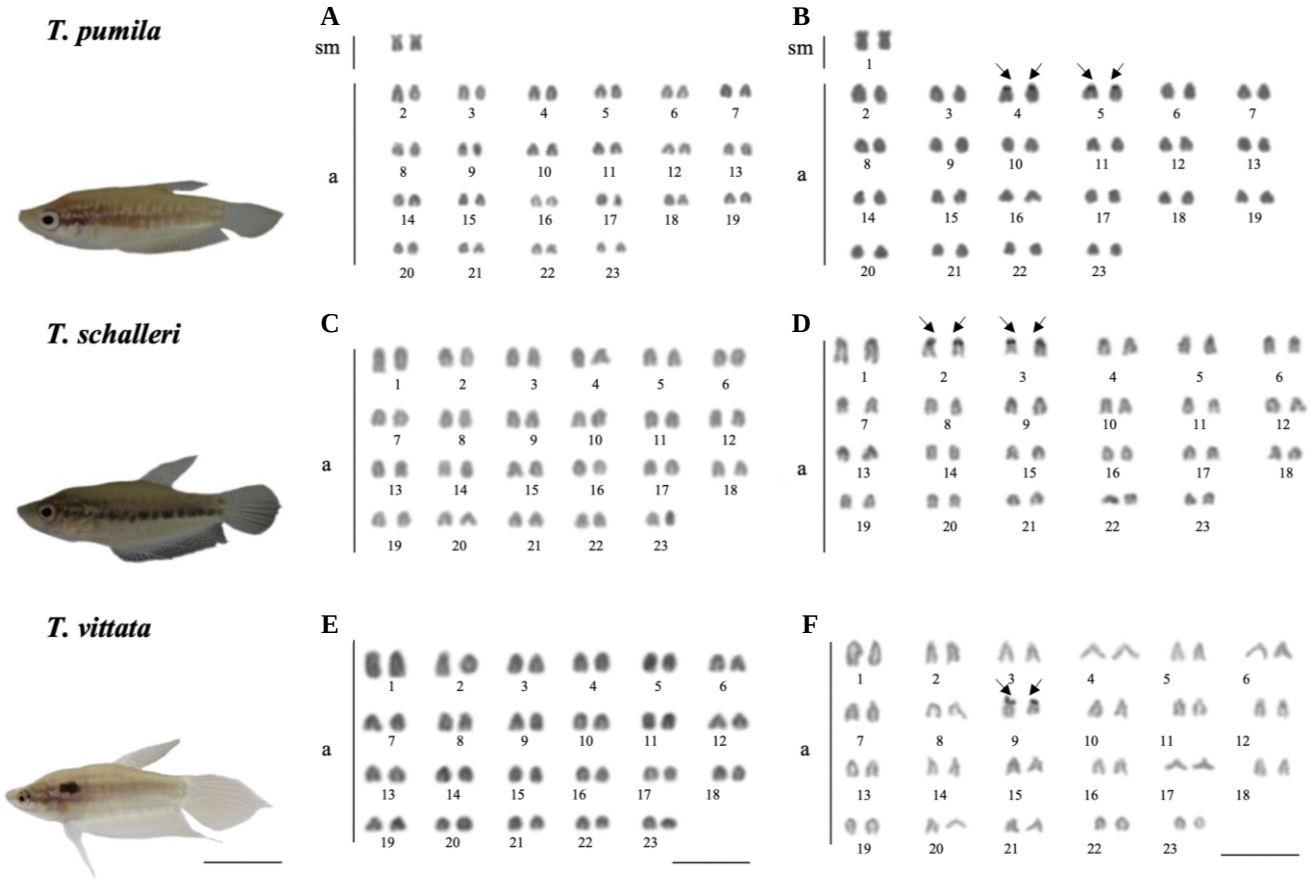


**Figure 1.** Pictures of analyzed *Trichopsis* (Anabantiformes, Osphronemidae) as *T. pumila*, *T. schalleri*, and *T. vittata* and their respective karyotypes by conventional Giemsa staining )A, C, E( and NOR banding )B, D, F(. Arrows indicate NOR-bearing chromosomes. Scale bars: 1.25 cm, 5 µm, and 5 µm, respectively

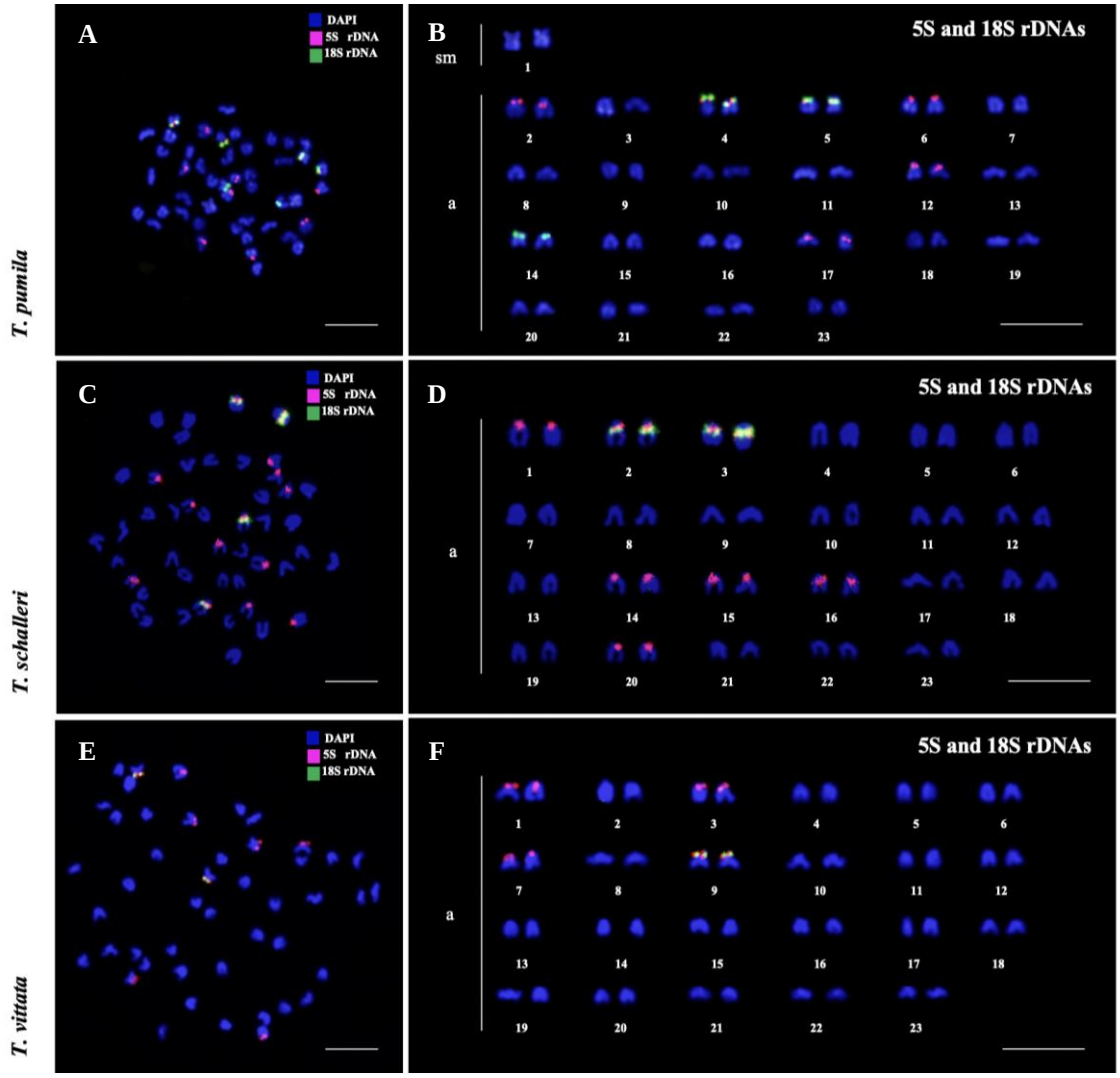


**Figure 2.** Mitotic metaphase chromosome plates and karyotypes of *Trichopsis*, *T. pumila* )A, B(, *T. schalleri* )C, D(, and *T. vittata* )E, F( with rDNA probes as 5S and 18S rDNA probes, on scale bars indicate 5 µm

The NORs-carrier chromosomes are taxonomic markers in several fish groups )Khakhong et al. 2014; Sochorová et al. 2018(. Apart from karyotype organization, *T. pumila*, like *T. schalleri*, has multiple Ag-NOR sites distributed across two chromosome pairs. That includes an interstitial pericentromeric position of pairs 4 and 5 and in pairs 2 and 3 in *T. schalleri*. When compared to single sites, such as in *T. vittata* pair 9. In several fishes, a single pair of NORs and multiple pairs of NORs were commonly seen in plesiomorphic conditions. That indicated an apomorphic or advanced state )Kumar et al. 2013; Supiwong et al. 2021(. Thus, the NOR site of *T. pumila* was suggested and supported the advanced character than that of *T. schalleri* and *T. vittata*. In previous reports, six species of family Osphronemidae, such as *Trichopodus trichopterus, Trichopodus leerii, Trichopodus microlepis, Trichopodus pectoralis, H. temminckii,* and *Belontia hasselti* have shown the location of Ag-NOR in only one pair of chromosomes )Arai 2011; Chaiyasan et al. 2021; Mingkwan et al. 2021; Supiwong et al. 2021(.

Ribosomal RNAs provide the foundation for life as we know it and are essential for the ribosome's functions in protein synthesis (Noller et al. 2017). They are shared by all eukaryotes and are regarded to be one of the oldest repetitive fractions since they are present in genomes in large numbers of copies and arranged in tandem arrays (Symonová et al. 2019). The rDNA genes are reported as conserved elements in the eukaryotic genome associated with intragenomic diversification )Rebordinos et al. 2013(. In each *Trichopsis* species analyzed here, there were distinct distribution patterns for both 5S and 18S rDNAs, with 18S motifs following the position of Ag-NOR. On the other hand, the 5S rDNA is distributed unevenly in each karyotype and is found in *T. schalleri*, *T. pumila*, and *T. vittata* on seven, six, and four chromosomes, respectively. Such distributional differences can also occur in closely related species and lead to sub-chromosomal background diversity linked to some speciation events (Symonová et al. 2016).

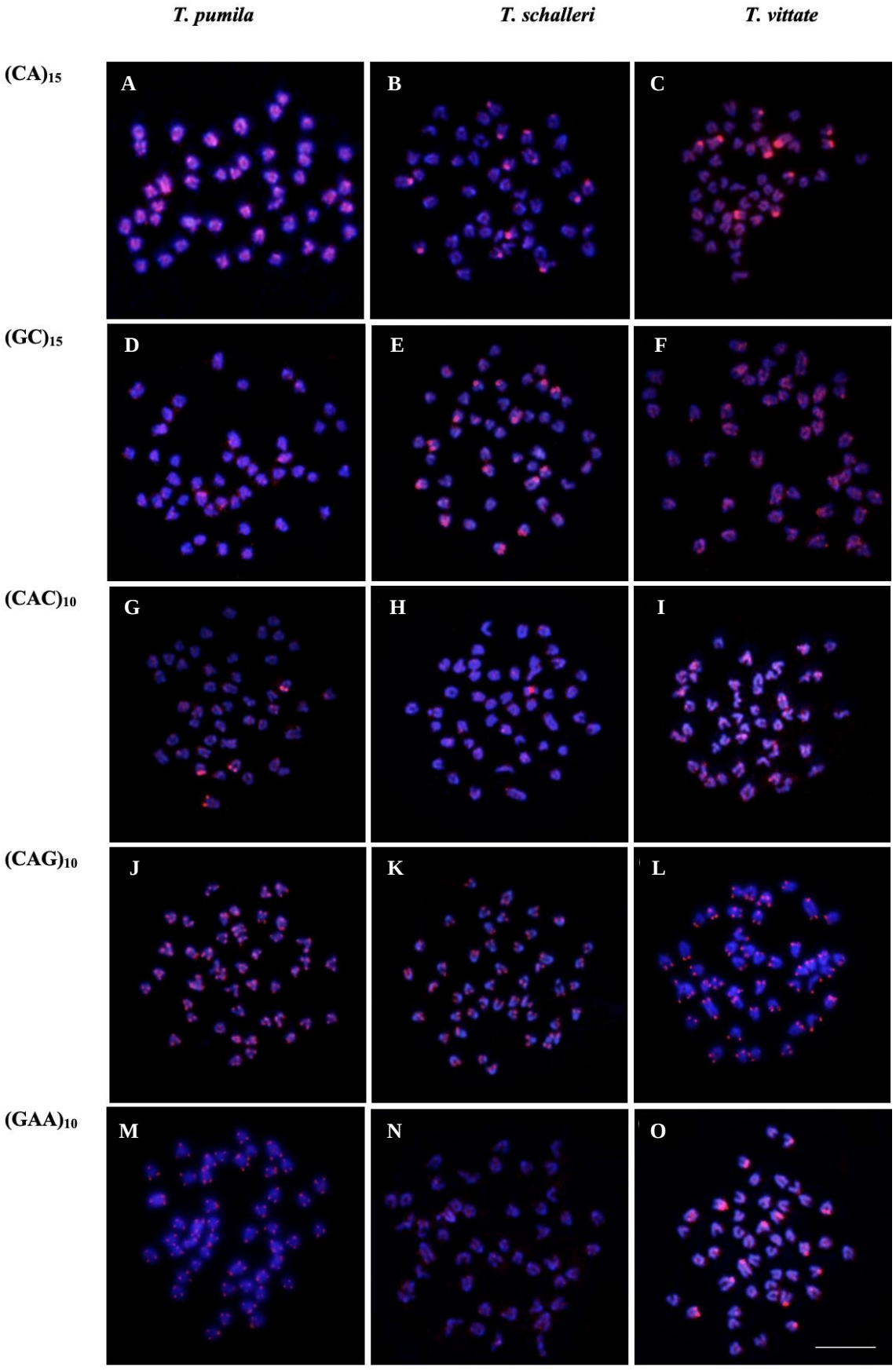


**Figure 3.** Mitotic metaphase chromosome plates of *Trichopsis*, *T. pumila* )A, D, G, J, M(, *T. schalleri* )B, E, H, K, N(, and *T. vittata* )C, F, I, L, O(, with di- and tri- microsatellites, d)CA(15, d)GC(15, d)CAC(10, d)CAG(10, and d)GAA(10 as probes. Scale bars indicate 5 µm

This has occurred especially in *Trichopsis* but not in the related genus *Belontia* and *Trichogaster*, which presents simple 5S and 18S rDNA sites (Pazza et al. 2009; Chaiyasan et al. 2021). The multiple 5S rDNA was hypothesized to be related to transposable elements )TEs( action in other fish groups. The moderate distribution of TEs is associated with karyotypic conservatism. Some classes of TEs could copy some sequences and move them to the other parts of the genome, affecting the recombination rate and spreading of 5S rDNA throughout chromosomes )Symonová et al. 2013; Moraes et al. 2017).

In fish genomes, microsatellites are typically found in the telomeric and centromeric regions of autosomal and sex chromosomes, which are together with other repetitive DNA sequences (Cioffi and Bertollo 2012). This pattern is also observed for the three gouramis species herein investigated. The result shares a similar distribution of $d(CAG)_n$ motifs in the telomeric region of all chromosomes, in addition to species-specific patterns for d)CA(15, d)GC(15, d)CAC(10, and d)GAA(10 probes. The position and number of microsatellite sequences on chromosomes may change across closely related fish species involved in recent speciation episodes, as reported for channid fish, naked catfishes, and silurids (Supiwong et al. 2014; Cioffi et al. 2015; Ditcharoen et al. 2020), and probable in *Trichopsis* species, as herein observed. The distribution of microsatellite motifs in fish genomes may be skewed toward certain noncoding areas, but it could also be related to the dispersion of rDNAs across chromosomes. This is because both repetitive motifs were observed in several fish species (e.g., Furo et al. 2017; Sassi et al. 2019; Ditcharoen et al. 2020; this study). The distribution pattern of d)CA(15 in *T. schalleri* and *T. vittata* was similar to general cases in previous studies, accumulating strongly in the centromeric region of some pairs. Whereas *T. pumila* seems to represent d)CA(15 throughout the whole genome in a consistent pattern with *B. hasselti* )Chaiyasan et al. 2021(, *Mystus* species )Yeesin 2021(, the Thai pufferfish *Pao cochinchinensis* )Pissaparn et al. 2020(, and Siluridae species )Ditcharoen et al. 2020). While on the d)GC(15 and $d(CAC)_{10}$ motifs revealed a dispersed pattern, with some accumulation in the centromeric and telomeric sites of a few chromosomes, especially in *T. schalleri* and *T. vittata*. In most centromeres and telomeres of all *Trichopsis* intense d)CAG(10 signals were found. Then, the d)GAA(10 also showed accumulation in centromeres and telomeres of some chromosomes, being more intense in *T. vittata* chromosomes. Several studies on fish have found repetitive DNA in the heterochromatic zones, such as telomeres, centromeres, and sex chromosomes (Supiwong et al. 2014). However, microsatellites could also be found in non-centromeric regions, usually associated with the location near or within genes )Getlekha et al. 2016b(. Repetitive DNA accumulation is known as the primary driving force in karyotype diversification related to speciation )Dernburg et al. 1996; Maneechot et al. 2016; Ditcharoen et al. 2020). On several fishes, the evolutionary karyotype change can be explained and estimated by studies, including: repetitive DNA mapping, remarkably microsatellites and ribosomal DNA elements )Getlekha et al. 2016a; Maneechot et al. 2016; Ditcharoen et al. 2019; Ditcharoen et al. 2020; Chaiyasan et al. 2021(.

In conclusion, our data from conventional staining, Ag-NORs, and mapping of repetitive sequences gave us a deeper understanding of the karyotypic structure and evolution of *Trichopsis* species. Despite having a shared 2n=46 diploid number, this study showed that the Ag-NOR sites and the distribution of repetitive sequences vary between species. Three Osphronemidae species contain at least three sex chromosomal systems, including ZZ/Z0 and XX/X0 in *Trichogaster lalius*, ZZ/ZW in *T. fasciata*, and ZZ/ZW in *B. hasselti* (Arai 2011; Chaiyasan et al. 2021). However, sex chromosomes were not found in all investigated *Trichopsis* species. Our further studies will focus on exploring the genetic diversity among these species using NGS (next-generation sequencing) approaches to obtain SNP markers, build a solid phylogeny for the group and discuss the chromosomal data under an evolutionary context.

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