



Morphological and Chromosomal Characterization of *Pethia setnai*- (Indigo Barb) from Western Ghats of India.

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Abstract: Morphological as well as Cytogenetical profiling was accomplished in vulnerable freshwater ornamental fish species, namely *Pethia setnai* (Indigo barb). The *Pethia setnai* comes under Order – Cypriniformes and Family- Cyprinidae. This species is generally found in west flowing rivers of western ghats of India. The live specimens of *Pethia setnai* were collected from the Kalna River in Sindhudurg district for the chromosomal as well as morphological analysis. Kidney and Gill cells from two male and two female fishes were used for the study. The chromosomal analysis was carried out by Conventional staining (Giemsa) technique. The cells showed diploid chromosome number i. e. $2n=52$ and reported 92 fundamental number (NF) in both the sexes. Karyologically, it composed of 20 metacentric, 8 sub-metacentric, 12 acrocentric and 12 telocentric chromosomes from 25 metaphasic field analysis. The Karyotype formula for the *Pethia setnai* is $2n$ (diploid) $52 = 20m+8sm+12a+12t$. The long arm chromosome length (L1), total length of chromosome (LT), Relative length (RL), Centromeric index (CI) of chromosome were calculated. The present investigation is the first report of karyological analysis of species *P. setnai*.

Keywords: Chromosomal Analysis, Morphology, *Pethia setnai*, Ideogram, Western Ghats

Introduction:

Western Ghats of India is one of the biodiversity hotspots in the world (Myers *et al.*, 2000; Sloan *et al.*, 2014). It has unique assemblages of animal and plant communities and endemic species (CEPF 2011). So far, 355 fish species are reported from Western Ghats including some of the new descriptions along with exotic species (Sajan, 2015)

The morphometric measurements & the meristic counts are considered as very simple and authentic method for the species identification, which can be signified as morphological systematics (Nayman, 1965). Morphometric features could be

interpreted as a powerful tool for assessing discreteness and relationship between the several taxonomic groups in fish biology (Melvin *et al.*, 1992; Nowak & Ohtsuki, 2008). Morphometric and meristic study on fishes will provide a potent tool for computing discreteness of the identical species, due to that reason, several ichthyologists used all these characters in their study for differentiation of fish species (Rohollah, 2013).

Hereditary characterization is a core piece for the conservation of fishes and it should be possible through cytogenetic, biochemical-hereditary and the most practical molecular-genetic techniques.

Chromosome is a genetic vehicle, which provides the fundamental material for genetic investigations, like gene maps construction and chromosomal models organization, investigation of role of gene (Gosden, 1994). Cytogenetic study in fish has developed speedily during last four decades along with the standardization of methods. The methods include both *in-vivo* as well as *invitro* treatments. In the *in-vitro* treatment, blood cells culture, abdominal fluid, scale epithelium or fin tip was used (Fan and Fox, 1990; Amemiya *et al.*, 1984; Gold *et al.*, 1990). Now a days, the importance of the chromosomal study has been increased, as this investigation helps in the areas of the systematics, aquaculture and mutagenesis and also in phylogenetic

relationship and hybridization etc. (Kligerman & Bloom, 1977). According to one report, out of total taxonomically reported fish fauna throughout the world, only 10 % of fish species have been studied cytogenetically (Barat and Sahoo, 2001).

Chhapgar (1992) first time described this species as *Puntius setnai* from Sanguem, Goa. Later on, Pethiyagoda *et al.*, (2012) established new '*Pethia*' genus based on the number of morphological, osteological & genetical characters. So, on the basis of this theory of Pethiyagoda, the genus of *Puntius setnai* is changed from *Puntius* to *Pethia* and now it is call it as *Pethia setnai*.

Table No.1. Review of Cytogenetic reports in *Pethia* genus from various localities

SR · N O.	SPECIES	2N	CYTOTYPE	NF	LOCALITI ES	REFERENCE
1	<i>Pethia setnai</i>	52	20m+8sm+12a+ 12t	92	Sindhudurg	Present Study
2	<i>Pethia conchoniuss</i>	50	4m+2sm+10a+2 4t	92	Nashik	Yadav & Muley 2019
3	<i>Pethia meingangbii</i>	50	24m+14sm+12a	88	Manipur	Sukham & et. al., 2015
4	<i>Pethia stoliczkanus</i>	50	22m+22sm+4st +2a	98	Thailand	Magtoon & Arai 1989
5	<i>Pethia cumingii</i>	50	18m+26sm/st+6 a	94	Asia	Taki & Suzuki 1977
6	<i>Pethia nigrofasciatus</i>	50	16m+34sm/st	10 0	Asia	Taki & Suzuki 1977
7	<i>Pethia ticto</i>	50	14m+22sm+6st +8t	86	West Bengal	Manna & Prasad 1973

Material and Methods:

Live species of *Pethia setnai* were collected from Kalna River in Sindhudurg district, Maharashtra. 05 fish species were

transferred and acclimatized in the laboratory aquarium for 10 to 15 days with aeration and fed ad libitum. The ethanol preserved specimens were used for the

morphometric and meristic character analysis. For morphometric analysis digital Vernier Calliper was used to record the measurements of different parts of fish body. The stereo microscope was used for the study of meristic characters in fish. The mitotic chromosomal preparation was obtained from the gill and liver cells samples by using the protocol of Khuda & Barat, 1987 and Chen & Ebeling, 1968 with slightly modification and observed under

the ZEISS trinocular microscope with 1000X magnification. The measurements or the length of the chromosomes were measured by using the ISIS- 5.5 Karyotyping software. In present study, the length of short arm (Ls) and long arm (L1) of chromosome, total length (LT) i.e. (LT= Ls+L1), Relative length (RL) and Centromeric index (CI) of chromosome were recorded.

Table no. 3.1 Fish sampling sites along with GPS locations and elevation.

Sr. No.	Name of district	Sampling Sites	Name of water bodies	Latitude	Longitude	Elevation in
1	Sindhudurg	Kalna	Kalna river	15°46'54.5"N	73°57'59.8"E	47
2	Sindhudurg	Kalna	Kalna river	15°46'55.9"N	73°57'59.3"E	47
3	Sindhudurg	Kalna	Kalna river	15°46'54.8"N	73°57'58.8"E	48

Results & Discussion:

For general appearance of fish body and its shape, see Plate no. I (A). During life, *Pethia setnai* has dull golden or silvery-grey color with two-three transverse black bands on body; anterior band situated between the dorsal and below the lateral line row encircling with 3rd and 4th scales of lateral line; the middle one is situated between posterior half of the dorsal fin base and 1 scale below lateral line encircling 9th - 11th lateral line scale; posterior band is in between the middle end of dorsal, caudal and the posterior half of the base of anal fin. Posterior and anterior bands are darker than that of the middle band. Lateral line is complete and shows 19-20 scales with 18-19 lateral line pores on it, barbels are absent, transverse scale rows consist of 4½|1|2½, 7 – 8 pre-dorsal, 10-11 pre-pelvic

and 15-16 pre-anal scales. Dorsal fin consists of unbranched 3 and branched 8 rays, the 3rd unbranched ray is strong and densely serrated with 10 serrated spines on it, pectoral fin consists of 1 unbranched and 11-12 branched fin rays, pelvic fin bears 1 unbranched and 7 branched fin rays and anal fin consist of 3 unbranched & 5 branched fin rays as shown table no. 1

Measurements of fish were made with the help of dial clipper i. e. Vernier Caliper by point-to-point to the near 0.1mm. The Standard length (SL) percent represented as subunit of body and Head length (HL) percent represented as subunit of head. 5 individuals of *P. lutea* from different localities were used for morphometric and meristic analysis as shown table no. table no. 2

Table no. 1 Meristics characters of *Pethia setnai*

Sr. No.	Meristics	Range
1	Lateral line scales	19-20

2	Transverse scale rows	(4½ / 1 /2½)
3	Pre-dorsal scales	7 - 8
4	Pre-pelvic scales	10 – 11
5	Preanal scales	15 - 16
6	Dorsal fin rays	iii8
7	Pectoral fin rays	i11 -i12
8	Pelvic fin rays	i7
9	Anal fin rays	iii5
10	Lateral line pore	18 -19
11	Dorsal fin ray serrae	11

Table no. 2 Morphometric characters of *Pethia setnai*

Sr. No.	Character	Mean ± SD (n=5)	Range
1	Total length (mm)	53.4 (1.6)	51.2 - 55.7
2	Standard length (SL, mm)	37.2 (2.2)	33.4 - 39.2
% SL			
3	Head length (HL)	26.6 (0.8)	25.9 - 28.3
4	Head depth	23.0 (1.2)	20.1 - 23.8
5	Head width	17.1 (4.1)	15.5 - 27.3
6	Body depth	37.7 (2.2)	33.6 - 40.2
7	Body width at dorsal fin origin	13.9 (0.4)	13.3 - 14.2
8	Body width at anal fin origin	4.0 (4.3)	07.5 - 8.7
9	Pre-dorsal distance	52.1 (1.2)	51.0 - 53.7
10	Dorsal to hypural distance	55.6 (1.4)	54.0 - 56.9
11	Pre-pelvic distance	48.6 (2.1)	45.6 - 51.5
12	Preanal distance	70.9 (1.7)	68.7 -72.7
13	Pre-pectoral distance	25.9 (3.0)	21.6 - 30.3
14	Dorsal fin length	30.2 (1.1)	29.3 - 31.6
15	Dorsal fin spine length	10.8 (11.6)	21.0 - 22.1
16	Length of dorsal fin base	18.5 (1.7)	16.4 - 20.4
17	Pectoral fin length	24.4 (1.5)	22.5 - 26.4
18	Anal fin depth	19.6 (2.3)	17.5 - 22.7
19	Caudal peduncle length	18.7 (1.7)	16.7 - 20.8
20	Caudal peduncle depth	16.1 (0.5)	15.4 - 16.5
% HL			
21	Head depth	86.6 (6.2)	74.4 - 92.0
22	Head width	64.3 (14.8)	55.7 - 100.7
23	Snout length	24.9 (2.5)	23.1 - 30.9
24	Eye diameter	36.4 (1.6)	33.5 - 38.3

25	Inter orbital width	43.6 (4.0)	39.5 - 49.0
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Specimens deposited in BNHS, Mumbai & ZSI Pune -

BNHS FWF-675, one specimen, 39.21mm, L_s, India, Maharashtra, Sindhudurg district, Kalna River, Kolzar, 15°46'55.7"N; 73°57'59.8"E; 47-meter elevation. Yadav R. B., 12-April-2016;

BNHS FWF-676, one specimen, 33.06mm, L_s, India, Maharashtra, Sindhudurg district, Kalna River, Kolzar, 15°46'55.7"N; 73°57'59.8"E; 47-meter elevation. Yadav R. B., 01-Feb-2016;

BNHS FWF-677, one specimen, 36.12mm, L_s, India, Maharashtra, Sindhudurg district, Kalna River, Kolzar, 15°46'55.7"N; 73°57'59.8"E; 47-meter elevation. Yadav R. B., 21-April-2017;

ZSI-WRC-P/5447, one specimen, 38.58mm, L_s, India, Maharashtra, Sindhudurg district, Kalna River, Kolzar, 15°46'55.7"N; 73°57'59.8"E; 47-meter elevation. Yadav R. B., 12-April-2017;

Chromosome analysis-

Table No. 3 - The mean of short arm chromosome length (L_s), long arm chromosome length (L₁), total length of chromosome (LT), Relative length (RL), Centromeric index (CI) from *Pethia setnai* (2n=52). All Measurements are in µm.

Chromosomes Pair	L _s	L ₁	LT	RL	CI	AR	Chromosome type
1	1.5794	1.7406	3.3200	0.0638	0.5243	1.1020	Metacentric
2	1.5754	1.6397	3.2150	0.0618	0.5100	1.0408	Metacentric
3	1.3443	1.3807	2.7250	0.0524	0.5067	1.0270	Metacentric
4	0.8968	1.4282	2.3250	0.0447	0.6143	1.5926	Metacentric
5	1.0273	1.2327	2.2600	0.0434	0.5455	1.2000	Metacentric
6	0.9032	1.0968	2.0000	0.0384	0.5484	1.2143	Metacentric
7	0.6788	1.1313	1.8100	0.0348	0.6250	1.6667	Metacentric
8	0.8056	0.9344	1.7400	0.0334	0.5370	1.1600	Metacentric
9	0.7419	0.9031	1.6450	0.0316	0.5490	1.2174	Metacentric
10	0.7081	0.8369	1.5450	0.0297	0.5417	1.1818	Metacentric
11	0.5488	1.6463	2.1950	0.0422	0.7500	3.0000	Sub-metacentric
12	0.7744	2.0006	2.7750	0.0533	0.7209	2.5833	Sub-metacentric
13	0.8707	1.3543	2.2250	0.0428	0.6087	1.5556	Sub-metacentric

Live fishes were collected from Kalna River, Sindhudurg for the karyological analysis. Gill and kidney cells from two male and the female fishes were used for the study. Conventional staining of both the cells showed diploid chromosomes 2n=52 and reported 92 fundamental number (NF) in both the sexes. Karyologically, it composed of 20 metacentric, 8 sub-metacentric, 12 acrocentric and 12 telocentric chromosomes from 25 metaphasic field analysis. The Karyotype formula for the *Pethia setnai* is 2n (diploid) 52 = 20m+8sm+12a+12t as shown in Plate no. 4.9 E- Spreading of metaphase and F – Karyotype of *P. setnai*. The details of chromosome calculations are shown in table no. 3. The present investigation is the first-time report of karyological analysis of species *P. setnai*.

14	0.7419	1.2581	2.0000	0.0384	0.6290	1.6957	Sub-metacentric
15	0.5497	1.3903	1.9400	0.0373	0.7167	2.5294	Acrocentric
16	0.4835	1.3215	1.8050	0.0347	0.7321	2.7333	Acrocentric
17	0.4846	1.1954	1.6800	0.0323	0.7115	2.4667	Acrocentric
18	0.5152	1.0948	1.6100	0.0309	0.6800	2.1250	Acrocentric
19	0.5159	1.0641	1.5800	0.0304	0.6735	2.0625	Acrocentric
20	0.3863	0.9337	1.3200	0.0254	0.7073	2.4167	Acrocentric
21	0.0000	2.1300	2.1300	0.0409	1.0000	0.0000	Telocentric
22	0.0000	1.9050	1.9050	0.0366	1.0000	0.0000	Telocentric
23	0.0000	1.8700	1.8700	0.0359	1.0000	0.0000	Telocentric
24	0.0000	1.7750	1.7750	0.0341	1.0000	0.0000	Telocentric
25	0.0000	1.7400	1.7400	0.0334	1.0000	0.0000	Telocentric
26	0.0000	0.9050	0.9050	0.0174	1.0000	0.0000	Telocentric

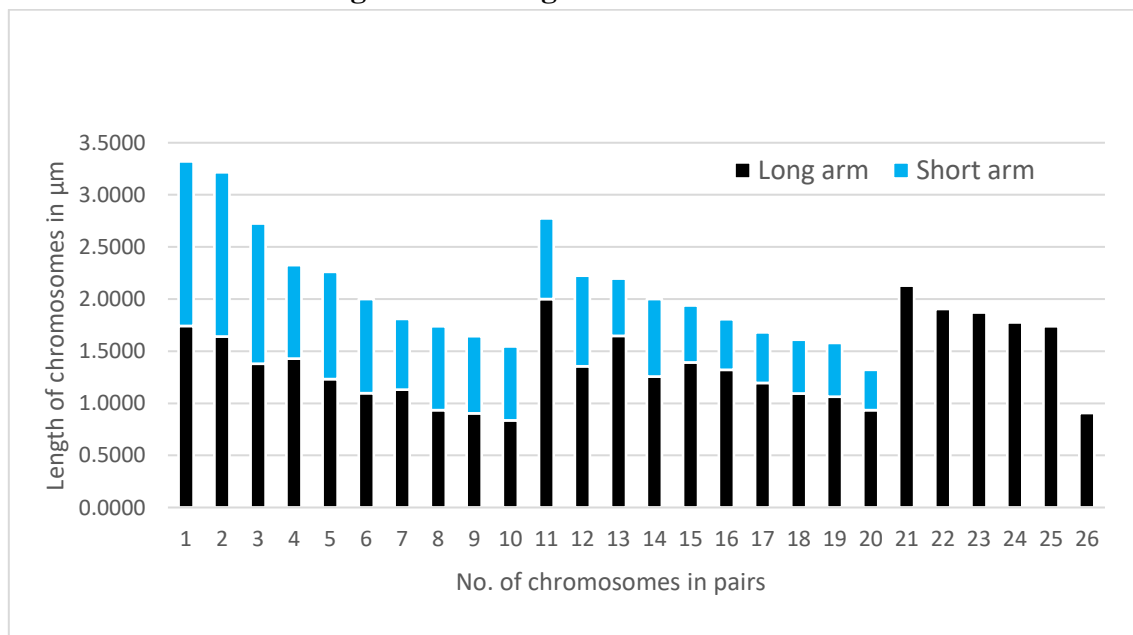
The largest chromosome observed in metacentric type of chromosome which is 3.32 μm , while the smallest chromosome is telocentric having 0.9050 μm .

Ideogram of *Pethia setnai* -

Ideogram represent lengths and shapes of chromosomes of *Pethia setnai*, $2n=52$ by conventional staining technique (Giemsa). The first 10 pairs represent metacentric chromosome, pairs 11th to 14th

is sub-metacentric chromosomes, pairs no. 15th to 20th shows acrocentric chromosomes and pairs 21th to 26th represent telocentric type of chromosomes. The ideogram (Fig. no. 1) and karyotype (Plate no. I- F) of *P. setnai* showed the gradually decreasing length of the chromosomes according to their type and chromosomes pairing pattern,

Fig. no. 1 – Ideogram of *Pethia setnai*



Pethia setnai is an endemic barb species to the Western Ghats of India (Yadav, 2003) In present study, it is first time reported from Sindhudurg district and distribution of this species was observed in Kalna River, Kolzar. As far as previous reports are concerned, it was observed in Tunga River basin, Chikmangalur-Karnataka by Atkore *et al.*, (2015), and Terekhol River- Madkhol, Maharashtra by Katwate *et al.*, (2013) [Plate no. I- C]. This species is reported as area specific in the present study, because they are only observed in specific stream of Sindhudurg district. At the time of collection, population of this fish was observed very less. According to IUCN Red list, it is a Vulnerable fish species because, there is no any proper information on its population. As far as morphological features are concerned, there is no any differences observed in fish, as they compare with the original description. Two to three transverse black bands and body has dull golden or silvery-grey colour during life, which is prominent characteristics of *Pethia setnai*.

The chromosomal analysis was carried out in this species for the first time, $2n=52$ number of chromosomes were reported in both the sexes with 95 number of fundamental number (NF). The chromosomal formula is composed of 20 metacentric, 8 sub-metacentric, 12 acrocentric and 12 telocentric chromosomes from 25 metaphasic field analysis. As far as earlier findings are concerned, the chromosomal reports from the same genus shows diploid $2n=50$ number of chromosomes [table no.1], but in *Pethia setnai* $2n=52$ chromosomes number was observed, that is one additional chromosomes pair is observed in this species. Krysanov & Golubtsov (2001) suggested that, change in chromosome

number may also occur due to aneuploidy and polyploidy. Aneuploidy is the gain or loss of one or more chromosome because of improper division of chromosomes at the time of meiosis stage.

We found 6 karyotypic records in Earlier literature of same genus but in different species. Yadav R. B. & Muley D. V. (2019) worked on chromosome analysis of *Pethia conchoniis* species and they record, $2n=50$ number of chromosomes. Sukham & *et. al.*, 2015., Magoon & Arai 1989, Taki & Suzuki 1977 and Manna & Prasad 1973 reported 50 number of chromosomes in *Pethia meingangbii*, *Pethia stoliczkanus*, *Pethia cumingii*, *Pethia nigrofasciatus* and *Pethia ticto* but show variation in karyotypic formula. Variation in chromosome size and type is due to intraspecific centric fusion, in that two telocentric chromosomes fuse together and form metacentric chromosome (Table no. 1). The result of $2n$ changes due to the centromeric fusion but fundamental number (NF) remains constant Q. Yu and R. Zhou (1996). Chromosome number and morphological variations can be found between different population of the same species or among different individuals of the same population or even in different cells of the same species (Debora & Luiz 2003).

Summery and Conclusion:

Pethia setnai is generally found in west flowing rivers of the western ghats of India. The two to three transverse black bands and has dull golden or silvery-grey color on body during life which is prominent morphological characteristics of *Pethia setnai*. In present study, $2n=52$ diploid no. of chromosomes was reported in same fish species from the Sindhudurg district, Maharashtra. The karyotypic

formula of species is composed of 20m+8sm+12a+12t. As far as earlier records are concern, it is found that, the present investigation is the first attempt to study chromosome in the *Pethia setnai*.

As far as previous records are conserved, all authors reported 50 number of chromosomes in the *Pethia* genus but in present study we reported 52 numbers of chromosome due to which there is need to do more advance study on the same species so as to understand its karyoevolution.

Acknowledgement.

The authors are thankful to DBT-IPLS Programme (Grant No. BT/PR 4572/INF/22/147/2012), New Delhi for financial support and encouragement. The authors are also thankful to Department of Zoology, Shivaji University Kolhapur and Thakur College of Science & Commerce, Kandivali (E) Mumbai for providing the laboratory facilities.

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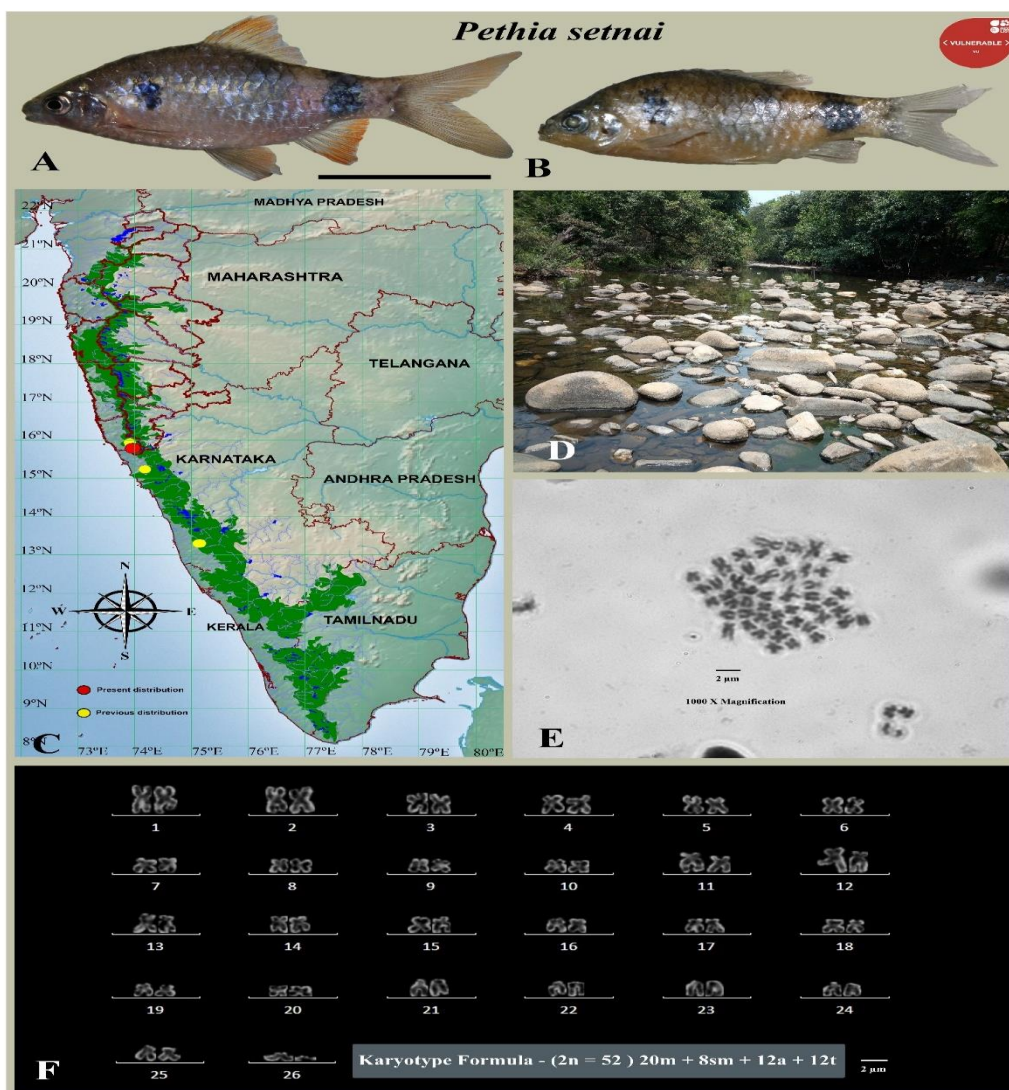
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PLATE NO. - I



A – Live specimen of *Pethia setnai*. Scale bar indicate 2 cm length of fish. B – Preserved specimen deposited in BNHS museum
 C – Map Showing distribution of *Pethia setnai* in Western Ghats of India.
 Red dot - present study record of specimen. Yellow dot - previous record of specimen. Green patch - range of Western Ghats.
 D – Collection site of *Pethia setnai* at Sindhudurg district, Kalna river, Kolzar village, Maharashtra.
 E – Metaphases of *Pethia setnai* with 1000 X magnification, scale bar indicates 2 µm length of chromosome.
 F – Karyotype of *Pethia setnai* by using conventional staining technique (Giemsa).