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Research Article

COMPARATIVE KARYOTYPE ANALYSIS OF SOME INDIGENOUS AND EXOTIC CICHLIDS OF SOUTH INDIA

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Cichlids are one of the most species rich fish families in the world. Researchers worldwide are attracted to cichlids due to their vast diversity. Fish cytogenetics plays an important role in classification and understanding evolution. The chromosome number and morphology of two indigenous Cichlids- *Etroplus suratensis* and *Etroplus maculatus*, and an exotic cichlid, *Pterohyllum scalare* are determined and compared in the present study. The method of Barreto Netto *et al*. and Nagpure *et al*. was followed for preparing chromosome spreads, which were photographed and measured. The chromosome compliment of *E. suratensis* consisted of 2n= 48 subtelo/acrocentric chromosomes. The chromosome compliment of *E. maculatus* consisted of 2n= 46 with a pair of metacentric chromosomes, which can be considered as marker chromosomes, and twenty two pairs of subtelo/acrocentric chromosomes. The chromosome compliment of *Pterohyllum scalare* consisted of 2n= 48 with 3 pairs of metacentric and 21 pairs of subtelo/acrocentric chromosomes. *E suratensis* retains the primitive ancestral karyotype and *E. maculatus* shows a derived, advanced karyotype. Derived karyotype occurs from ancestral karyotypes as a result of chromosomal rearrangements- fusions and inversions.

INTRODUCTION

The family Cichlidae is comprised of over 2000 species of fishes found in fresh as well as brackish water habitats in Africa, South America, North America and Southern parts of India. The natural distribution of the cichlids is centred on Africa, Latin America and Madagascar, with a few species native to south Asia and the Middle East (Snoeks, 2004). Cichlids have always attracted researchers mainly due to their great adaptive radiation, species richness and their importance as food fish (Barluenga *et al*., 2006). The study on fish chromosomes has received considerable attention in recent years because of their importance in classification, evolution, heredity (Gold *et al*., 1990), fish breeding, rapid production of inbred lines and cytotaxonomy (Kirpichnikov, 1981). Basic information on the number, size and morphology of chromosomes are needed to undertake genetic investigations such as hybridization and chromosomal manipulation in fish (Khan *et al*., 2000). It is also is one of the powerful genetic markers in the taxonomic identification of evolutionary significant units (Moriiz *et al*., 1987). Though it has the limitation to draw absolute conclusions about phyletic relationships from the karyological comparison of different species, yet the results provided by such reports have a major role in the understanding of evolutionary pathways within distinct groups (Affonso *et al*., 2007). In the present study, the karyotype analyses of two indigenous Cichlids, *Etrolus suratensis* and *Etroplus maculatus*, and an exotic ornamental cichlid, *Pterophyllum scalare* are performed to study the variation in chromosome number and morphology between them.

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MATERIALS AND METHODS

The two indigenous Cichlids were collected from Vellayani Lake (8.4344°N, 76.9917°E), Thiruvananthapuram and the exotic cichlid was collected from local aquarium shop in Thiruvananthapuram. Karyotyping was performed following the in vivo chromosome preparation method of Barreto Netto *et al*. (2007) and Nagpure *et al*. (2007), with appropriate modifications as per laboratory conditions. The slides were then screened for good chromosome spreads under a light microscope (LABOMED model CXL MONO) using 100X oil immersion objective. Good quality chromosome spreads were photographed by MagCam digital camera using MagVision software. The karyotyping was done by using SmarType software. The lengths of chromosomes were measured and ANOVA was performed by SPSS software. The chromosomes were then classified based on their arm ratio as per the technique mentioned by Levan *et al*. (1964).

RESULTS AND DISCUSSION

Good quality chromosome spreads were selected for further analyses. (fig.1). The diploid number of *Etroplus suratensis* was found out to be 48 (fig.2). All chromosomes were subtelo/acrocentric (fig.3). The total length of the chromosome ranges between 3.434±0.003µm to 1.756±0.001µm (table.1). Diploid number of *E. maculatus* was found to be 46 (fig.4), consisting of two metacentric and forty four subtelo/acrocentric chromosomes (fig.5). The first pair of metacentric chromosomes are distinctly larger than the rest of the chromosomes. The total lengths of the chromosome range between 1.687±0.202µm and 7.971±0.099µm (table.2). The diploid number of *Pterophyllum scalare* was found out to be 48 (fig.6), consisting of six metacentric and forty two subtelo/acrocentric chromosomes (fig.7). The total arm length ranged from 11.954±0.066µm to 3.759±0.010µm. (table.3). The dipoid number of *E. suratensis* was in conformity with earlier studies done by Natarajan and Subrahmanyam (1974); Rishi and Singh (1982); Das (1990) and Antony and Kumari (2016). The karyotype formula determined in the present study showed a difference from the formula determined by Natarajan and Subrahmanyan (1974). The diploid number of *E. maculatus* coincides with the earlier works of Natarajan and Subrahmanyan (1974); Das (1990) and Poletto *et al.* (2010). A variance in diploid number was established in the work of Antony and Kumari (2016). The karyotype formula of *E. maculatus* reveals two large metacentric chromosomes which can be considered as marker chromosomes, which is also observed by Das (1990) and Poletto *et al*. (2010), though the karyotype formula determined by them differ from that obtained in the present study. Simiar diploid number for *Pterophyllum scalare* was obtained in the studies of Thompson (1979); Krichana *et al*. (1996); Nascimento *et al*. (2006) and Poletto *et al.* (2010). The karyotype formula determined for *P. scalare* in the present study shows dissimilarity to the karyotype formulae determined by all the previous studies except that of Poletto *et al.* (2010).

Several authors have reported a diploid number of 48 in many cichlids. In study of Thompson, (1979), 31 out of the 41 species under study showed a diploid count of 48. It is evident from the literatures that most of the representatives from the subfamilies Cichlinae, Astronotinae, Geophaginae and Cichlasomatinae display a diploid number of 48. Works of Roncati *et al*. (1996) in *Crenicichla sp*., Oyhenart Perera *et al*. (1975) in *Crenicichla sexatilis* and Feldberg and Bertollo, (1985) in *Crenicichla lepidota*, *C. semifasciata* and *C. lacustris*, Farias *et al*. (1999) in *Crenicichla cf. saxatilis*, Thompson (1979) in *Cichla temensis*, *Crenicichla lucius*, *C. notophthalmus* and *C. strigata*, Martins *et al*. (1995) in *Crenicichla niederleinii*, Alves *et al*. (1999) in *Crenicichla reticulata*, Fenocchio *et al*. (1994) and Salgado *et al*. (1994) in *Crenicichla sp*. reveals the diploid number of 48 in the subfamilly Cichlinae. Likewise, works by Krichanã *et al*. (1996) in *Astronotus crassipinnis*, Zahner (1977) and Scheel (1973) in *Astronotus ocellatus*, Feldberg and Bertollo (1985) in *Chaetobranchopsis australis* indicates that 48 is the most prevalent diploid number in the subfamily Astronotinae. Studies by Thompson (1979) in *Acarichthys heckelii*, *Geophagus brasiliensis*, *Geophagus surinamensis* and *Satanoperca jurupari*, Salgado *et al*. (1994) in *Geophagus altifrons*, Peixoto and Erdtmann (1988) in *Gymnogeophagus gymnogenys*, Feldberg and Bertollo (1985) in *Gymnogeophagus balzanii*, Peixoto and Erdtmann (1988) in *G. rhabdotus*, Farias *et al*. (2000) in *Satanoperca acuticeps*, Salgado *et al.* (1994), Moreira Filho *et al*. (1980) and Mendonça *et al*. (1999) in *Satanoperca jurupari*, and Martins *et al.* (1995) in *Satanoperca pappaterra* reveals 48 as the diploid number in the subfamily Geophaginae.

It can be understood that 48 is the diploid number shown by most of the representatives of the subfamily Cichlasomatinae from the studies done by Thompson (1979) in *Aequidens metae*, *Amphilophus citrinellus*, *A. macracanthu*, *Archocentrus nigrofasciatus*, *Archocentrus centrarchus*, *Archocentrus septemfasciatus*, *Cichlasoma beani, Cichlasoma bimaculatum*, *Cichlasoma octofasciatus*, *Cichlasoma trimaculatus*, *Herichthys cyanoguttatus*, *Herichthys labridens*, *Herichthys minckleyi*, *Herotilapia multispinosa*, *Hypselecara coryphaenoides*, *Mesonauta festivus*, *Neetroplus nematopus*, *Parachromis dovii*, Martins-Santos *et al*. (1990) in *Aequidens plagiozonatus*, Scheel (1973) in *Aequidens pulcher*, Salgado *et al*. (1994) in *Cichlasoma amazonarum*, Farias *et al.*(2000) in *Aequidens tetramerus*, Nishikawa *et al*. (1973) in *Amphilophus citrinellus*, Zahner (1977) in *Amphilophus macracanthus*, Hinegardner and Rosen (1972) in *Amphilophus macracanthus*, Loureiro and Dias (1998) in *Cichlasoma paranaense*, Santos *et al*. (2001) in *Mesonauta insignis* and Ráb *et al*. (1983) in *Nandopsis tetracanthus*.

It is often postulated by many workers that the modal diploid number of the subfamily Pseudocrenilabrinae, the subfamily that constitutes the African cichlids, is 44 (Feldeberg *et al.*, 1990). However, some individuals; *Neolamprologus leleup*, *Oreochromis alcalicus* and *Pelvicachromis pulche* shows a diploid number of 48 as evident from the work of Post (1965). The existence of diploid number 46 is not a discrete phenomenon as observed in *Etroplus maculatus* in the present study. The Pseudocrenilabrinae representative, *Melanochromis auratus* (Thompson, 1981), the Cichlinae representative *Crenicichla sp* (Rezende *et al*., 1996), the Geophaginae representatives *Apistogramma agassizii* (Thompson, 1979), *Apistogramma ortmanni* (Thompson, 1979), *Apistogramma steindachneri* (Zahner, 1977) *Apistogramma trifasciata* (Roncati *et al*., 2000) and *Dicrossus filamentosus* (Thompson, 1979) show diploid their number as 46.

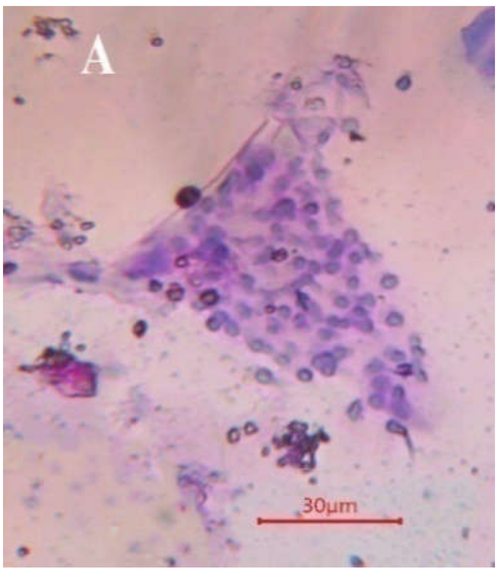
The presence of 2 pairs of large meta/submetacentric chromosomes is a remarkable character shown by *E. maculatus* in the present study. Such a trend is also exhibited by *Geophagus brasiliensis* and *Gymnogeophagus balzanii* as is evident from the works of Feldberg and Bertollo, (1985) and Roncati *et al*. (1996). Both of these fishes belong to Geophagnae subfamily. A study by Salgado *et al*. (1994) also revealed similar chromosome morphology in *Cichlasoma amazonarum*. A study by Valente *et al.* (2012) revealed the presence of 2 remarkably large metacentric chromosomes that are larger than the remaining chromosomes in *Laetacara araguaiae* belonging to the cichlinae subfamily. Poletto *et al*. (2010) identifes a similar condition in the Psedocrenilabrinae subfamily in *Oreochromis aureus, O. tanganicae* and *O. Niloticus*.

Karyotype formula similar to that of *P. scalare* is exhibited by many cichlids. In the Cichlinae family, *Crenicichla lacustris*, *C. lepidota*, *C. notophthalmus*, *C. reticulata*, *C. semifasciata*, *C. strigata* and *C. vittata* exhibits a similar karyotype formula as clearly understood from the works of Thompson (1979) and

Feldberg and Bertollo (1985). Thompson (1979) identifies *Astronotus ocellatus* and *Acarichthys heckelii* to possess a similar karyotype from Astronotinae and Geophaginae subfamilies respectively. *Geophagus brasiliensis* posses the same karyotype formula as shown in the studies of Brum *et al*. (1998) and Quijada and Cestari (1998).

It is generally accepted that the ancestral or primitive karyotype of teleost consists of 48 chromosomes. According to Feldberg *et al.* (2003), the standard karyotype of 48 monoarmed chromosomes is found more often among the cichlids. Although the diploid chromosome number ranges from 2n=32 to 2n=60 chromosomes, more than 60% of the species present a karyotype with 2n = 48. A bimodal distribution of diploid numbers is evident and related to the geographic distribution of the cichlid species; African cichlids have a modal diploid number of 2n = 44, with a variation from 32 to 48 chromosomes, while Neotropical cichlids have a modal diploid number of 2n= 48, with a variation from 38 to 60 chromosomes. Mayers and Roberts (1969) and Ohno (1970) states that primitive fishes have higher chromosome number and fewer metacentric chromosomes than their more advanced relatives. The decrease in number of chromosomes and variation in biarms indicates a derived karyotype. This could be derived from ancestral karyotype as a result of an evolutionary step in which all or a large percentage of chromosomes underwent centric fusions (Robertsonian fusion) (Thompson, 1979) and pericentric inversions. This would result in numerous metacentric chromosomes and in turn a reduced diploid number.

Subtelocentric/acrocentric chromosomes constitute a major portion of the karyotype of the three cichlids in the present study. On the other hand, a comparative examination of the karyotype formulae of the three cichlids reveals that the indigenous cichlids show less number of metacentric chromosomes than that of the exotic cichlids. Therefore, the indigenous cichlids can be assumed to be primitive than the exotic cichlid. Among the two indigenous cichlids, *E. suratensis* can be considered as primitive species, while *E. maculatus* as an advanced species.



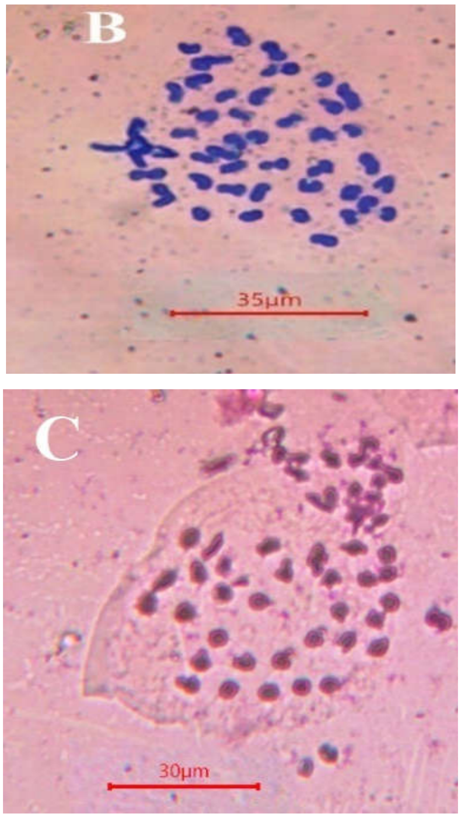


Fig 1 Chromosome spreads of A) *Etroplus suratensis*, *B) Etroplus maculatus,* C) *Pterophyllum scalare*

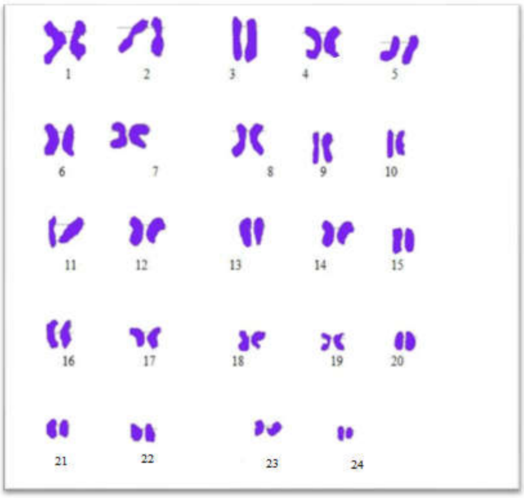


Fig 2 Karyotype of *Etroplus suratensis.* (Chromosome number 1 to 24- st/a) st/a: subtelo/acrocentric

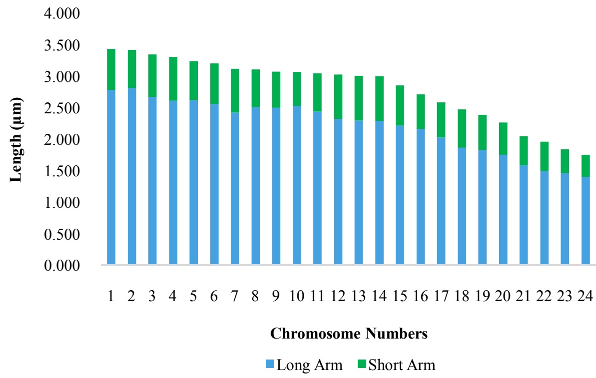


Fig 3 Ideogram showing different types of chromosomes in *Etroplus suratensis*

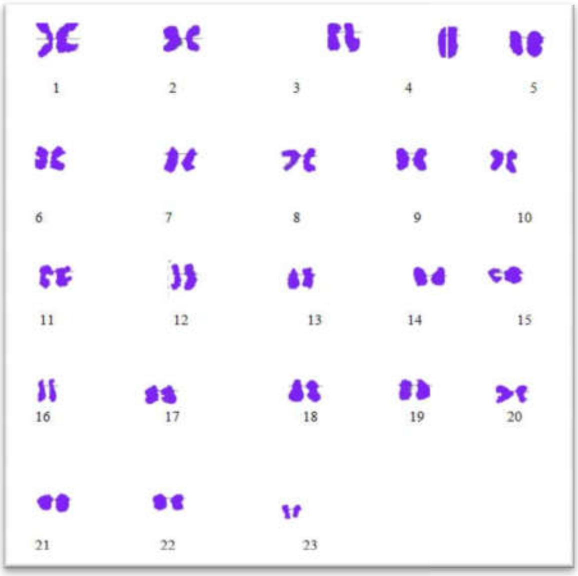


Fig 4 Karyotype of *E. maculatus* (Chromosome number 1 and 2-m and 3 to 23 - st/a) m: metacentric st/a: subtelo/acrocentric

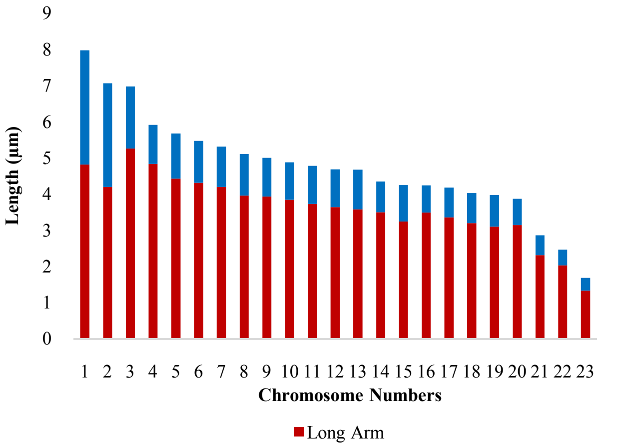


Fig 5 Ideogram showing different types of chromosomes in *E. maculatus*

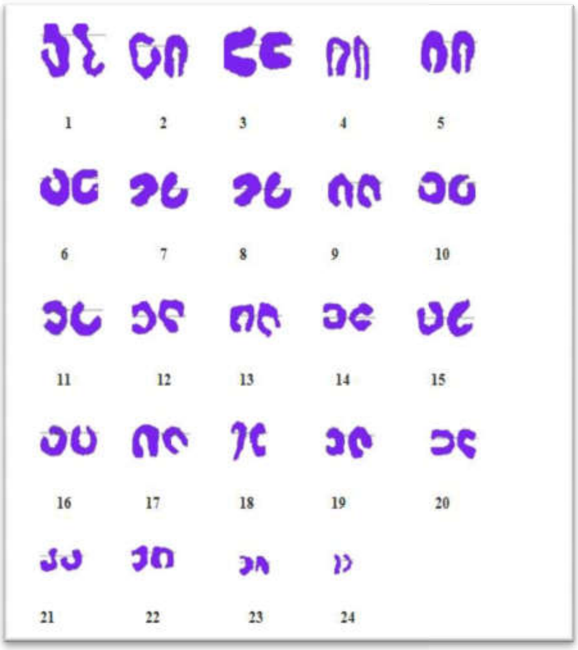


Fig 6 Karyotype of *P. scalare*(Chromosome metacentric, chromosomes 3, 7, 13, 14, 15,17- m; chromosomes1,2, 4-6,8-12, 16, 18-24- st/a)) m: metacentric st/a: subtelo/acrocentric

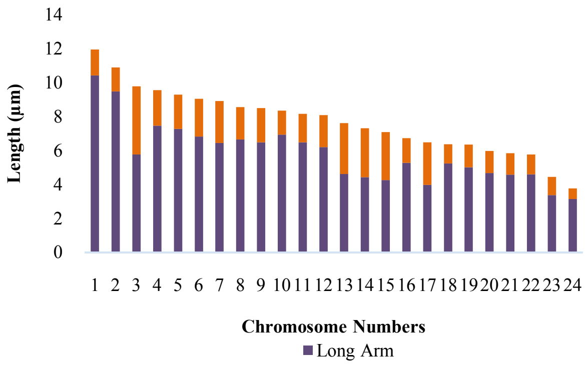


Fig 7 Ideogram showing different types of chromosomes in *P.scalare*

Table 1 Measurements and classification of chromosomes of *Etroplus suratensis*

Chromosome No.	LA(µm)	SA(µm)	TL(µm)	AR(µm)	Type
1	2.781±0.005	0.653±0.007	3.434±0.003	4.261±0.049	st/a
2	2.812±0.010	0.607±0.004	3.418±0.006	4.633±0.050	st/a
3	2.672±0.006	0.674±0.002	3.346±0.004	3.965±0.020	st/a
4	2.609±0.005	0.696±0.001	3.305±0.004	3.747±0.015	st/a
5	2.621±0.055	0.616±0.001	3.238±0.054	4.253±0.099	st/a
6	2.559±0.002	0.645±0.004	3.203±0.002	3.968±0.026	st/a
7	2.426±0.002	0.694±0.004	3.120±0.001	3.494±0.021	st/a
8	2.511±0.009	0.599±0.001	3.110±0.009	4.194±0.010	st/a
9	2.497±0.001	0.577±0.001	3.074±0.002	4.327±0.009	st/a
10	2.529±0.007	0.537±0.001	3.066±0.006	4.713±0.019	st/a
11	2.436±0.074	0.613±0.066	3.049±0.008	4.018±0.590	st/a
12	2.320±0.051	0.706±0.042	3.027±0.008	3.295±0.279	st/a
13	2.296±0.001	0.712±0.006	3.009±0.005	3.224±0.029	st/a
14	2.293±0.003	0.705±0.004	2.999±0.001	3.251±0.022	st/a
15	2.217±0.001	0.635±0.003	2.852±0.002	3.492±0.016	st/a
16	2.162±0.028	0.548±0.002	2.710±0.029	3.949±0.046	st/a
17	2.028±0.041	0.557±0.002	2.585±0.042	3.639±0.065	st/a
18	1.868±0.040	0.607±0.002	2.475±0.042	3.076±0.057	st/a
19	1.828±0.022	0.562±0.001	2.390±0.021	3.256±0.043	st/a
20	1.755±0.064	0.513±0.001	2.268±0.063	3.421±0.130	st/a
21	1.585±0.053	0.464±0.010	2.048±0.063	3.414±0.041	st/a
22	1.495±0.053	0.468±0.011	1.963±0.042	3.201±0.193	st/a
23	1.465±0.003	0.376±0.023	1.841±0.021	3.903±0.234	st/a
24	1.404±0.006	0.353±0.006	1.756±0.001	3.980±0.087	st/a

LA: long arm, SA: short arm, TL: total length, AR: arm ratio, st/a: subtelo/acrocentric.

Table 2 Measurements and classification of *E. maculatus* chromosomes

Chromosome No.	LA (µm)	SA (µm)	TL (µm)	AR (µm)	Type
1	4.820±0.301	3.151±0.396	7.971±0.099	1.552±0.271	M
2	4.201±0.183	2.859±0.048	7.060±0.136	1.470±0.089	M
3	5.256±0.027	1.720±0.010	6.976±0.017	3.056±0.034	st/a
4	4.835±0.096	1.080±0.009	5.915±0.092	4.477±0.110	st/a
5	4.431±0.161	1.249±0.042	5.680±0.122	3.553±0.249	st/a
6	4.316±0.132	1.153±0.021	5.469±0.112	3.746±0.183	st/a
7	4.197±0.063	1.118±0.057	5.315±0.010	3.763±0.254	st/a
8	3.962±0.029	1.153±0.021	5.114±0.008	3.437±0.088	st/a
9	3.932±0.037	1.074±0.002	5.006±0.039	3.660±0.028	st/a
10	3.847±0.015	1.031±0.012	4.877±0.026	3.733±0.030	st/a
11	3.731±0.115	1.053±0.023	4.783±0.097	3.546±0.182	st/a
12	3.645±0.027	1.041±0.031	4.686±0.006	3.502±0.127	st/a
13	3.584±0.023	1.089±0.006	4.674±0.018	3.290±0.038	st/a
14	3.504±0.196	0.844±0.211	4.347±0.022	4.346±1.153	st/a
15	3.242±0.017	1.007±0.002	4.248±0.016	3.220±0.022	st/a
16	3.489±0.041	0.756±0.042	4.245±0.001	4.627±0.300	st/a
17	3.361±0.047	0.825±0.000	4.185±0.047	4.076±0.057	st/a
18	3.199±0.033	0.836±0.027	4.035±0.006	3.832±0.168	st/a
19	3.101±0.037	0.874±0.010	3.975±0.038	3.549±0.060	st/a
20	3.148±0.017	0.723±0.039	3.871±0.022	4.361±0.248	st/a
21	2.313±0.056	0.553±0.021	2.866±0.036	4.191±0.254	st/a
22	2.035±0.064	0.430±0.021	2.465±0.043	4.749±0.370	st/a
23	1.337±0.204	0.350±0.022	1.687±0.202	3.836±0.683	st/a

LA: long arm, SA: short arm, TL: total length, AR: arm ratio, m: metacentric st/a: subtelo/acrocentric

Table 3 Measurements and classification of *P. scalare* chromosomes

Chromosome No.	LA(µm)	SA(µm)	TL(µm)	AR(µm)	Type
1	10.424±0.110	1.531±0.044	11.954±0.066	6.815±0.262	st/a
2	9.484±0.069	1.400±0.084	10.884±0.029	6.792±0.442	st/a
3	5.765±0.078	4.012±0.073	9.777±0.083	1.437±0.038	M
4	7.468±0.018	2.092±0.008	9.561±0.012	3.569±0.022	st/a
5	7.265±0.040	2.031±0.015	9.295±0.025	3.578±0.046	st/a
6	6.829±0.018	2.216±0.012	9.045±0.008	3.082±0.025	st/a
7	6.445±0.106	2.468±0.030	8.912±0.076	2.612±0.076	M
8	6.652±0.017	1.906±0.041	8.558±0.035	3.492±0.079	st/a
9	6.479±0.011	2.012±0.011	8.491±0.021	3.221±0.014	st/a
10	6.925±0.020	1.419±0.023	8.344±0.042	4.881±0.067	st/a
11	6.478±0.117	1.685±0.133	8.163±0.047	3.864±0.354	st/a
12	6.202±0.245	1.875±0.219	8.077±0.026	3.352±0.561	st/a
13	4.607±0.469	3.000±0.421	7.607±0.048	1.574±0.411	M
14	4.427±0.043	2.878±0.070	7.305±0.030	1.539±0.051	M
15	4.265±0.464	2.811±0.655	7.076±0.192	1.591±0.472	M
16	5.271±0.194	1.459±0.049	6.730±0.146	3.617±0.258	st/a
17	3.968±0.209	2.513±0.281	6.481±0.082	1.597±0.245	M
18	5.244±0.016	1.128±0.041	6.372±0.030	4.654±0.178	st/a
19	5.010±0.034	1.333±0.051	6.344±0.020	3.762±0.164	st/a
20	4.666±0.051	1.297±0.117	5.964±0.067	3.618±0.347	st/a
21	4.571±0.019	1.278±0.016	5.850±0.021	3.576±0.053	st/a
22	4.602±0.035	1.163±0.006	5.765±0.029	3.959±0.051	st/a
23	3.376±0.025	1.064±0.003	4.440±0.027	3.173±0.020	st/a
24	3.151±0.037	0.608±0.047	3.759±0.010	5.205±0.442	st/a

LA: long arm, SA: short arm, TL: total length, AR: arm ratio, m: metacentric st/a: subtelo/acrocentric.

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