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Karyological Studies of *Garra trewavasae* Monod 1950 from Jos Plateau, Nigeria.

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Abstract

Garra trewavasae Monod 1950 is a Cyprinid fish known only from the Jos Plateau, Nigeria. However, there are controversies over its taxonomic placement. Unfortunately, there is no information about its karyotype which is an important data useful to assess its taxonomic status. In this study we provide for the first time the karyotype of G. trewavasae using the conventional Giemsa staining technique. The diploid number (2n) was represented by 50 chromosomes and this corresponds to the diploid number reported for all other African Garra species. The number of chromosome arms (NF) was 85 and it falls within the range reported for African Garra species. The karyotypic formula was 1M + 9m + 3sm + 2st + 35t. However, unlike other African Garra species, a big unpaired metacentric chromosome which corresponds to the X chromosome according to earlier studies on African Garra species was detected. The sex of the species studied is therefore a male.

Key words: *African Garra species, Chromosome, Cyprinidae, Jos Plateau, Karyotype.*

Introduction

The genus *Garra* is one of the genera under the tribe Labeonini of the family Cyprinidae and its distribution range includes tropical and subtropical highlands of the Old World from Borneo through Central Asia and India to the Democratic Republic of the Congo and Senegal basin (Menon, 1964). The taxonomic placement of the genus *Garra* within the tribe Labeonini and also the internal groupings within the genus has been a subject of reviews (Reid 1982, 1985; Rainboth 1991, 1996; Zhang *et al*., 2000, Zhang and Chen 2004; Stiassny and Getahun, 2007). However, recently, Yang *et al*. (2012) and Yang *et al*. (2015) found the genus to be non-monophyletic.

Stiassny and Getahun (2007) recognised 17 African *Garra* species. The species are *G. duobarbis* Getahun 2000, *G. regressus* Getahun 2000, *G. quadrimaculata* (Rüppell, 1835), *G. hindi* (Boulenger, 1905), *G. blanfordii* (Boulenger, 1901), *G. geba* Getahun 2000, *G. dembeensis* (Rüppell 1835), *G. congoensis* Poll 1959, *G. ornata* (Nichols & Griscom 1917), *G. ethelwynnae* Menon 1958, *G. aethiopica* (Pellegrin, 1927), *G. makiensis* (Boulenger 1904), *G. ignestii* (Gianferrari 1925), *G. tana* Getahun 2000, *G. dembecha* Getahun 2000, *G. allostoma* Roberts 1990, *G. lancrenonensis* Blache & Miton 1960. The recognition of these seventeen African *Garra* species by Stiassny and Getahun (2007) was largely based on the taxonomic review of African *Garra* species by Getahun (2000) where the author resurrected *G. aethiopica*, *G. blanfordii* and *G. hindii* and synonimized *G. trewavasae* Monod 1950 and

G. tibanica Trewavas, 1941 with *G. ornata* and *G. quadrimaculata* respectively. However, Fricke *et al*. (2019) retained the species status of *G. trewavasae* implying that there is still controversy on the taxonomic status of the fish. Pending further assessment, this study recognises the species status of *G. trewavasae,* an African species of *Garra* reportedly found only on the Jos Plateau, Nigeria.

Fishes are known to have extensive chromosomal polymorphism and this has made their chromosomal information an important tool in taxonomy and analysis of evolutionary patterns within their taxa. However, unlike the Asian *Garra* species, the African *Garra* species have received very little cytogenetic attention. Available literature records to our knowledge show that only the karyotype of three African *Garra* species (*G*. dembeensis, *G. makiensis*, *G*. *quadrimaculata*) have been assessed (Krysanov and Golubstov, 1993). This study is therefore, the first assessment of the karyotype of *Garra trewavasae* towards a better understanding of its taxonomic placement.

Materials and Methods

Live samples of *G. trewavasae were* collected from Tahoss River, Jos, Plateau State, Nigeria. The chromosome extraction procedure was initiated by injecting the fishes with 0.02 ml/gram of colchicine from a stock of 0.01% wt/vol colchicine. The injection was made intra-peritoneal at the muscle mass of the base of the dorsal fin. The specimens were sacrificed four hours after the colchicine treatment and the gills were excised. The tissues excised were placed in a hypotonic solution of 0.56% KCl for 30 minutes, fixed in Carnoy's fluid (3 ethanol : 1 acetic acid). The cell suspension was dropped onto microscope slides at a height to promote good spreading of the cells, air-dried and stained with 6% Giemsa stain for twenty-five minutes. Images were captured using an Omax G013055005 model microscope fitted with an Omax A 35140U model camera; and processed using GNU Image Manipulation Program (GIMP 2.10.8). The length of each chromosome and idiogram were determined using Karyotype 2.0 (Altinordu *et al*., 2016)). The chromosomes were classified as metacentric (m) or submetacentric (sm), telocentric (t) or subtelocentric (st) (Levan *et al.*, 1964).

Results

The chromosome spread obtained for *Garra trewavasae* is shown in Plate 1 while Plate 2 shows the karogram with diploid chromosome number of 2n = 50. However, the fundamental number of the autosomal arms (NFa) is 85 and the karyotype formula is 2n = 1M + 9m + 3sm + 2st + 35t. The chromosome nomenclature in Table 1 shows that chromosomes 9, 7, 6, 5, and 4 are metacentric while chromosome 1 is submedian and chromosome 2 subterminal. On the other hand, chromosomes 3, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24 are telocentric. The length of the diploid set of chromosomes ranges between 0.6 µm and 0.17 µm for the longest and the shortest chromosomes respectively.

The karyotype of *Garra trewavasae* shows that the chromosomes can be grouped into four classes on the basis of size. The first class (Group A) comprises the large-sized chromosomes consisting of six pairs, chromosome 1 to 6. The second class (Group B) consists of medium-sized chromosomes consisting of six pairs, chromosomes 7 to 12. The third class (Group C) is made up of the small- sized chromosome consisting of twelve pairs, chromosomes 13 to 24. The fourth class (Group D) is the sex chromosomes, comprising only chromosome 25.

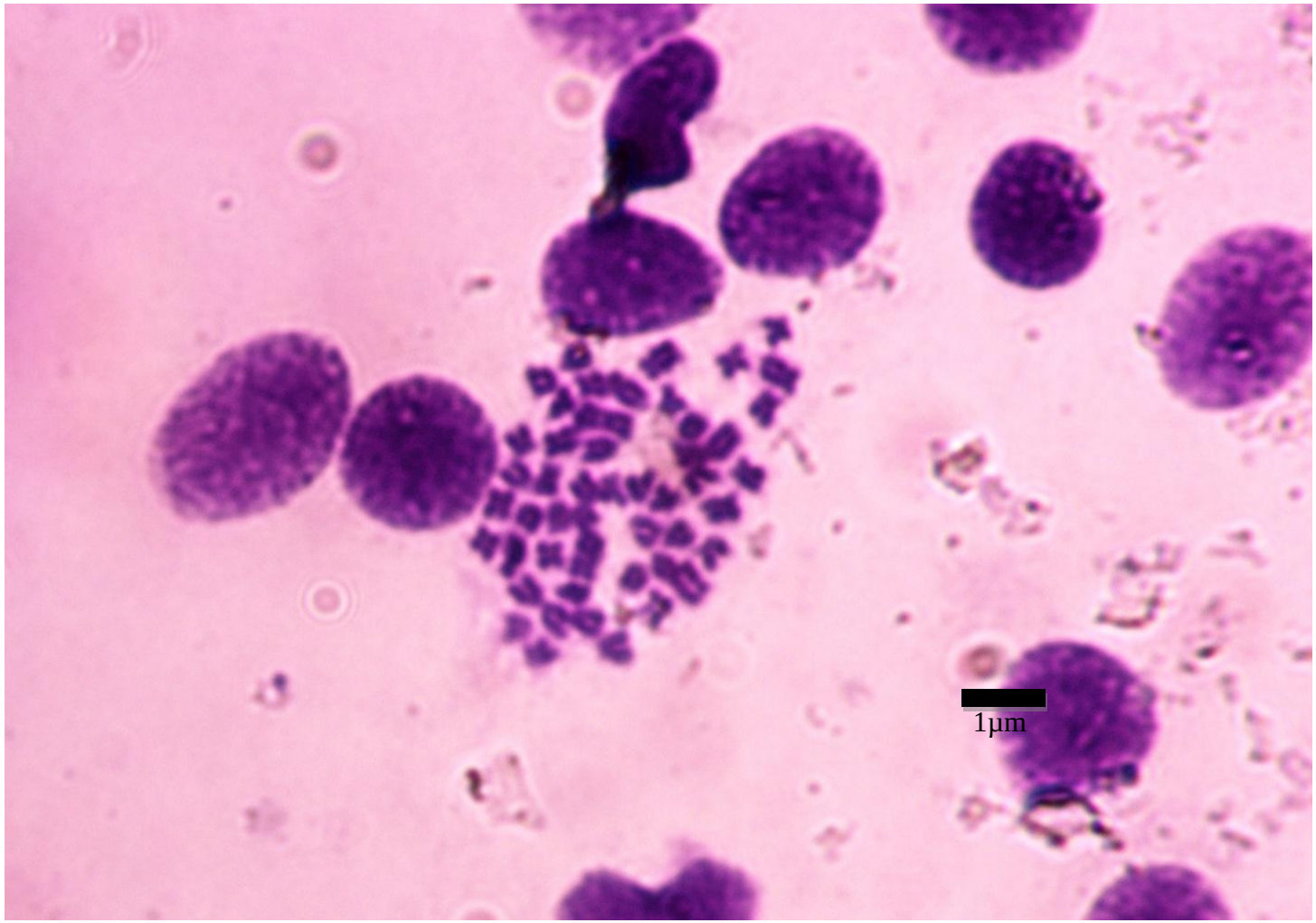

Plate I: Mitotic Metaphase chromosome spread of *Garra trewavasae*, 2n = 50

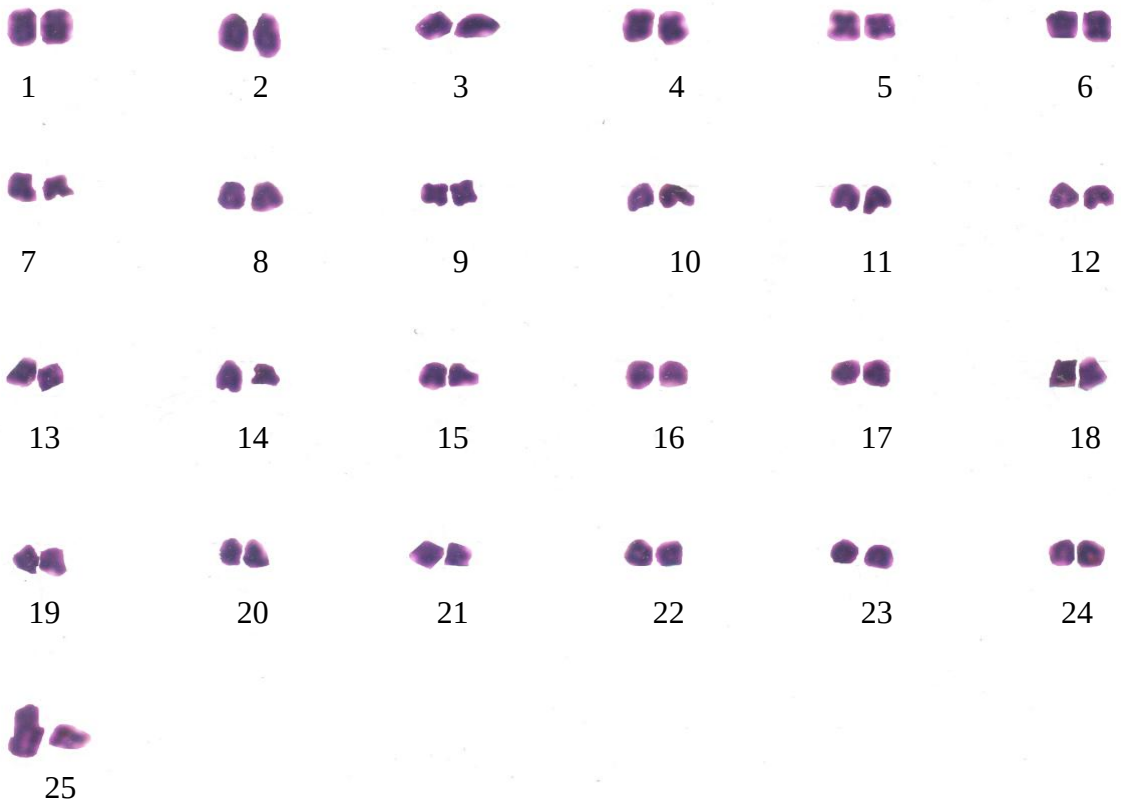

Plate 2: Karyotype of *Garra trewavasae*

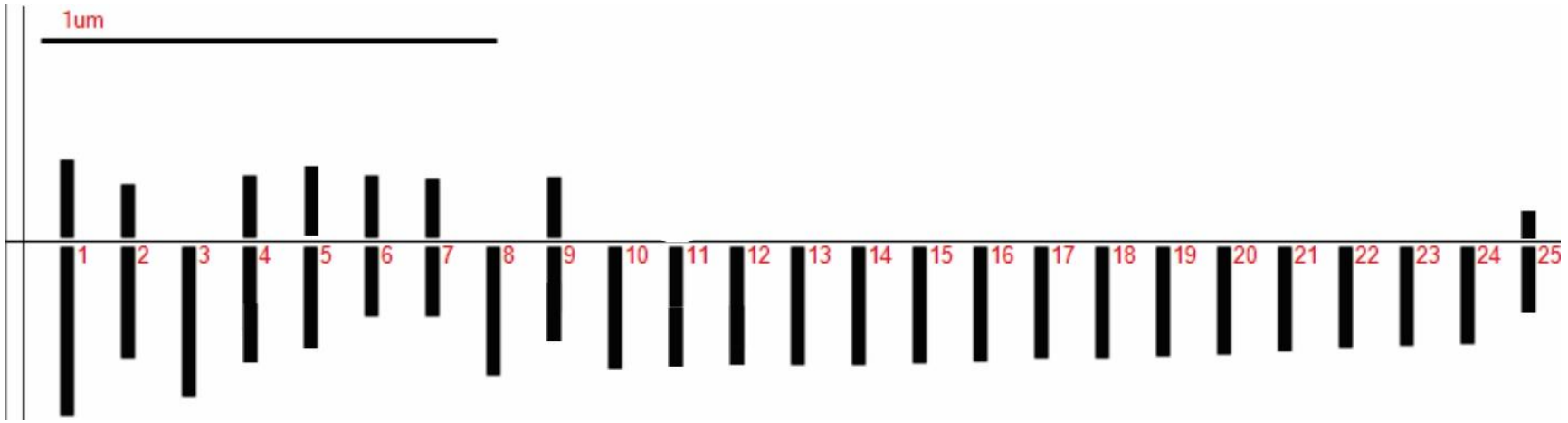

Fig. 1: An idiogram of the karyotype of *Garra*. *trewavasae* showing the morphology of the chromosomes

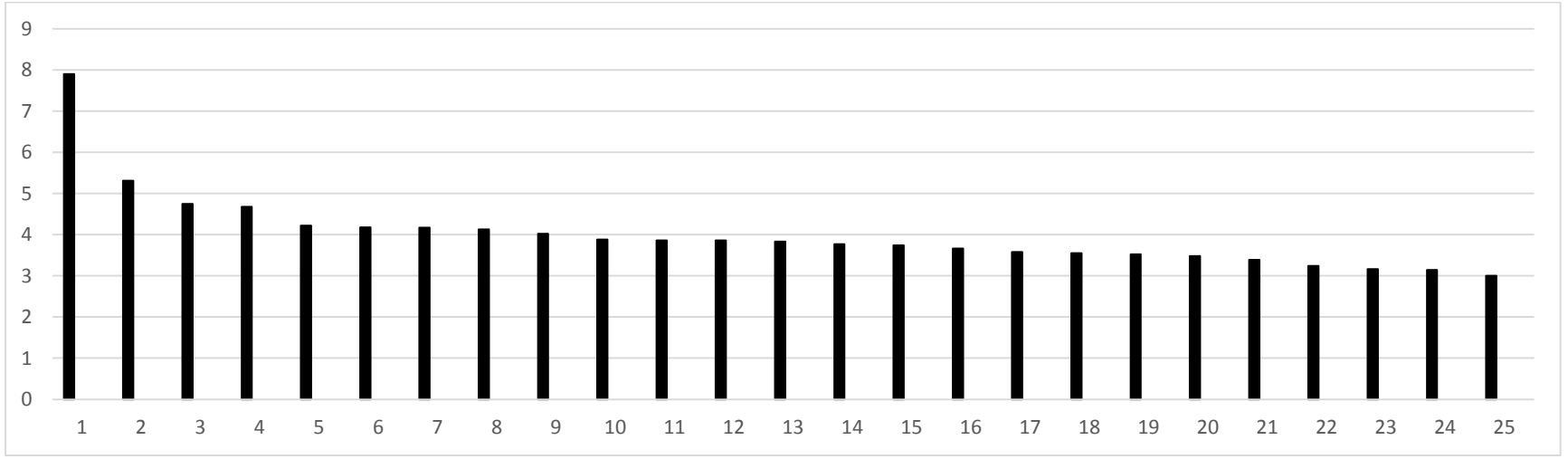

Fig. 2: Relative length of the chromosomes of *Garra trewavasae* showing the size variation

Table 1: The chromosome measurement and nomenclature of *Garra trewavasae*

	Measurement (µm)			**Relative length (%)**						
Chromosome No.	**Short arms (µm)**	**Long arm l (µm)**	**Total Length c (µm)**	**Short arms' (%)**	**Long arm l' (%)**	**Total length c' (%)**	**Centromeric Index (CI 1)**	**Arm Ratio (L/S %)**	Nomenclature	
1	0.37	0.17	0.54	5.39	2.51	7.9	31.73	2.15	submedian	sm
2	0.24	0.12	0.36	3.57	1.74	5.31	32.75	2.05	subterminal	st
3	0.33	0	0.33	4.75	0	4.75	0	3252.31	terminal	t
4	0.18	0.14	0.32	2.67	2.01	4.68	42.92	1.33	median	m
5	0.29	0	0.29	4.22	0	4.22	0	2890.35	median	m
6	0.15	0.14	0.29	2.2	1.98	4.18	47.41	1.11	median	m
7	0.15	0.13	0.29	2.25	1.92	4.17	46	1.17	median	m
8	0.28	0	0.28	4.13	0	4.13	0	2826.47	terminal	t
9	0.14	0.13	0.28	2.07	1.95	4.02	48.55	1.06	median	m
10	0.27	0	0.27	3.88	0	3.88	0	2655.82	terminal	t
11	0.13	0.13	0.26	1.94	1.92	3.86	49.69	1.01	terminal	t
12	0.23	0.04	0.26	3.31	0.55	3.86	14.18	6.04	terminal	t
13	0.26	0	0.26	3.83	0	3.83	0	2622.56	terminal	t
14	0.26	0	0.26	3.77	0	3.77	0	2580.43	terminal	t
15	0.26	0	0.26	3.74	0	3.74	0	2559.45	terminal	t
16	0.25	0	0.25	3.66	0	3.66	0	2505.48	terminal	t
17	0.25	0	0.25	3.58	0	3.58	0	2454.12	terminal	t
18	0.24	0	0.24	3.55	0	3.55	0	2427.39	terminal	t
19	0.24	0	0.24	3.52	0	3.52	0	2408.27	terminal	t
20	0.24	0	0.24	3.48	0	3.48	0	2381.43	terminal	t
21	0.23	0	0.23	3.39	0	3.39	0	2318.25	terminal	t
22	0.22	0	0.22	3.24	0	3.24	0	2219.78	terminal	t
23	0.22	0	0.22	3.16	0	3.16	0	2164.53	terminal	t
24	0.21	0	0.21	3.14	0	3.14	0	2149.57	terminal	t
25	0.21	0	0.21	3	0	3	0	2050.58	submedian	sm

*(CI 1) = 100s/c and (CI 2) = 100l/c.

The morphology of the chromosomes in form of an idiogram is presented in Fig. I, showing five metacentric chromosomes, one submetacentric chromosome, one subterminal chromosome and seventeen telocentric chromosomes. The graph of the relative length of the chromosomes is presented in Fig. 2.

Discussion

The diploid number of 2n = 50 determined for the karyotype of *Garra trewavasae* in this study corresponds well to the diploid number reported for all other African (Krysanov and Golubtsov 1993) and most of the Asian *Garra* species (Khuda-Bukhsh *et al*., 1980; Khuda-Bukhsh and Barat 1987; Yu *et al*., 1987; Sahoo *et al*., 2007). The diploid chromosome number of most of the *Garra* species that have been reported is 2n=50 and only few species have diploid numbers that are less than 2n =50 (Gozukara and Cavas, 2004; Esmaeili *et al*., 2009). The diploid chromosome numbers in Cyprinidae is known to be conserved and it is suggested that this might be an evidence that the chromosome number of Cyprinidae are not susceptible to chromosome structural changes such as fissions or fusions which normally precipitate changes (non-polyploid) in chromosome number.

The morphology of *G. trewavasae* chromosomes ranges gradually from a median to a nearly terminal position which is the pattern that has been observed in most Cyprinid species (Rab *et al*., 1995; Naran, 1997). Most of the chromosomes in this study were telocentric and similar pattern was found for *G. gotyla gotyla* by Sahoo *et al*. (2007) and the author showed that Robertsonian rearrangements as well as pericentric inversions were the main rearrangements responsible for such high number of telocentric chromosome. The number of chromosome arms (NF) of *G. trewavasae* (85) falls within the range (82–88) reported for African *Garra* species (Krysanov and Golubtsov 1993). A large submetacentric chromosome defined as a marker chromosome for the *Garra* genus (Sahoo *et al*., 2007) was also found in the karyotype of this study. This large submetacentric chromosome pair is very stable within the genus (Khuda-Bukhsh and Barat 1987; Khuda-Bukhsh 1984; Nagpure *et al*. 2006; Gozukara and Cavas 2004).

On the other hand, no karyological indication was found for sex chromosome dimorphism in African *Garra* species (Krysanov and Golubtsov 1993) and some Asian species of *Garra* (Esmaeili *et al*., 2009) although, heteromorphic chromosomes have been observed between the sexes in many fish species belonging to different systematic groups (Devlin & Nagahama 2002). However, this study found a big unpaired submetacentric chromosome which corresponds to the X chromosome according to earlier studies on African *Garra* species by Gorshkova *et al*. (2012). The biggest unpaired submetacentric chromosome observed indicate that the karyotyped species are males and it can be referred to as a presumable case of male heterogamety.

G. trewavasae therefore, share karyological features with conspecific from Africa and Asia but present a unique identity (possibly for now) in terms of sex chromosome when compared with conspecific from Africa.

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