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Introducing the first karyotype from the genus *Paramerina* of the subfamily Tanypodinae (Diptera: Chironomidae)

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Abstract

The first karyotype of an unspecified species ($2n=12$) from the genus *Paramerina* from the subfamily Tanypodinae collected from the south Indian geographical localities is presented. A tentative polytene chromosomal map was prepared and also the karyotype was prepared from somatic metaphase complement. The basic chromosomal number has been deduced from the karyotypes. Chromosomal descriptions also involve the patterns of C-bands and NOR localization. Head capsule morphology was used to arrive at the genus level identifications. Morphology of the shortest pompon-like chromosome has been discussed.

Keywords: Tanypodinae, karyotype, Polytene chromosomes, differential replication, pompon

Introduction

The subfamily Tanypodinae under the family Chironomidae includes about 673 species of 58 genera worldwide. The genus *Paramerina* is distributed worldwide comprising about Twenty-eight species [29, 4, 25, 1, 31, 36, 30, 10, 5, 24, 26, 11] four species are known to be described in India [4, 11].

The polytene chromosomes of the subfamily Tanypodinae range from $2n=6$ to $2n=16$ in number [27]. The salivary glands possess cells of different sizes that contain thin long globular polytene chromosomes that have low degree of polyteny, appearing as loose amorphous structures hence stain poorly, which makes their banding pattern indistinct and difficult to analyze [43]. Certain common features in some species of Tanypodinae are indistinct centromeres, absence of nucleolus and balbiani rings, pompon-like smallest chromosome containing a nucleolus very often, differentiated sex chromosomes with XX (females) and XY (males) and tight or no conjugation of homologous chromosomes [34, 35, 27]. Prevalence of acrocentric chromosomes, which is a typical of primitive groups found common in Tanypodinae [33, 36].

The karyological data of 17 species from 8 genera of subfamily Tanypodinae of the world fauna has been reviewed [27]. The karyotype of an unspecified species of genus *Anatopynia* based on the material collected from India has been reported [28]. The cytological descriptions of only a few genera in the subfamily Tanypodinae have been attempted due to the difficulty to prepare chromosomes and poor morphology and stainability of polytene chromosomes. The karyological studies of the genus *Paramerina* have not been attempted yet. In this respect the present report is the first chromosomal description from the genus *Paramerina* from a few locations in southern part of India. We describe the polytene and mitotic karyotype of the unspecified species of the genus, which henceforth designated as *Paramerina sp.*

Larvae of subfamily Tanypodinae possess characteristic strong premental structures such as Ligula and Paraligula, which may be light in color or darkly pigmented and Pecten hypopharynges [13] that are useful in genus level identifications and to a certain extent species level identification as well.

Materials and methods

The fourth instar larvae were collected from Sulikere lake (12.9716° N, 77.5946° E), Jnanabharathi campus pond (12.9529° N, 77.5165° E) (Bengaluru); Gudipalli (13.7846° N, 77.7982° E), (Chikballapura, karnataka) by scooping the mud with an enamel pan between 0-1 meter depth at the margin of pond /lake sites. The head capsule of each larva was separated from the body with a scalpel, cleared in warm 10% KOH and mounted ventral side up on a

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microscope slide on a drop of DPX. A 20 mm coverslip was centered over the head capsule and allowed to settle. A gentle pressure was applied to the coverslip to aid in flattening. Few identification keys were utilized for species identification based on certain available morphology traits^[9, 30, 7].

The Polytene chromosomes from salivary gland nuclei of fourth instar larvae were prepared based on the conventional Lacto-aceto Orcein temporary squashing technique^[23]. The mitotic and meiotic chromosome preparations from Neuroblast and gonadal cells respectively were made following the air drying procedure^[6] and were later stained by 5% diluted Giemsa solution. For staining of constitutive heterochromatin, the conventional procedure of Sumner^[38] was employed. Silver Staining was carried out according to the modified technique of Howell & Black^[12].

The chromosomal preparations obtained were subsequently subjected to microphotography using Zeiss Axioskop 2 plus microscope fitted with AxioCam MRc camera and Axiovision software. The photographs were processed in Adobe Photoshop version 7. For karyotype preparation Levan *et al.*,^[18] technique was followed in the laboratory. For cytophotomaps preparation a comprehensive description of mapping and nomenclature following Martin^[21] was adopted with relevant modifications. The total chromosome length (l) of each chromosome in the complement was measured using ImageJ software and relative length of each chromosome in the total complement length (L) was calculated (Table 1 & 2).

Results

Head capsule morphology

The slide mounted head capsule showed a fork shaped Ligula (figure 1a, b), brownish yellow colored with 5 darker teeth; one central and two laterals at each side. The central tooth is shorter than the laterals that are bent outwards. The paraligula (figure 1b) at both the sides found to have two projections; the outer being longer than the inner. Pecten epipharynges ((figure 1b) consisted of 14- 15 yellow teeth that are bent outwards; lateral ones are stouter and longer than the central ones. Mandible (figure 1a) was identified yellow colored with apical tooth black, basal tooth brown with 2-3 proximal small teeth.

Karyotype characteristics

The somatic mitotic chromosomal karyotype prepared from the neuroblast cells consisted of diploid number $2n=12$ (figure 1j,k), revealing 2 pairs of metacentric, 3 pairs of acrocentric and 1 pair of telocentric chromosomes. The somatic metaphase chromosomal karyotype (figure 1k) was prepared by arranging the chromosome pairs in the decreasing order of their total length. Majority of the mitotic metaphase plates stained with the conventional Giemsa technique alone revealed the heterochromatic spots in the centromeric region (figure 1j). The smallest pair appears very dark indicating heterochromatinization probably representing the sex chromosomes. These heterochromatic spots were remained as such after Silver nitrate staining (figure 1h, i). The C-bands appeared very feeble localized in the centromeric regions and telomeric regions of chromosome pair 1 and the probable sex chromosome pair (figure 1f). The telomeric asynapsis was evident in Diplotene and late pachytene stages especially of chromosomes 3 and 4 and these chromosomes in Metaphase seem to contain the satellites (figure 1c). A dense deposition of representing NOR was observed in interphase cells after silver nitrate staining (figure 1g, i). Sex chromosomes were found to be differentiated as XX and XY (figure 1d, e).

By taking 5 well spread metaphases and 5 polytene complements from the different populations the chromosome measurements were performed. The relative length of tiny arm differs between the polytene and mitotic metaphase complements. It was found out to be 4 times higher in metaphase chromosomes than the polytene chromosomes (tables 1 and 2).

Polytene Chromosomes

The polytene chromosomal complement (figure 2a) revealed 6 individual chromosomal elements. The polytene karyotype (figure 2b) consisted of 2 metacentric, 3 acrocentric and a telocentric chromosomal elements. The chromosomal arms combinations were designated as AB, CD, E, F, G and H (figure 2b). The centromeric region is flanked by blocks of heterochromatin (figure 2 a,b) making the centromeric region very distinct to identify. Although the occurrence of asynapsis was found to be very common in the polytene chromosomes of Tanyptodinae, no such prevalence was observed in the polytene chromosomes of *Paramerina sp.*, while it is seen in the meiotic metaphases in the chromosomes 3, 4 and 5 (figure 1c, d, e). The sixth chromosome in the polytene complement was found to be highly heteromorphic in morphology (figure 1l) that behaved differently in polytene and somatic cells. No polymorphism was noticed in the polytene chromosomes of the populations examined. The polytene chromosomal arms were found to display features and banding sequences as described below,

Chromosome 1, the longest component of the complement composed of arm A and B. Arm A begins with a light stained tip, the succeeding portion is darkly stained (divisions 1-5). The zone 6 is occupied by NOR (6 a-d). The proximal portion of arm B begins with a doublet of thick bands (7c-d) followed by lightly stained region. The succeeding portion includes sets of prominent dark bands interspersed with pale stained zones and the distal end is lightly stained.

Chromosome 2 is composed of arm C and D. Proximal end of the arm C is light stained followed by consecutive series of doublets in the zones 1 to 3. Following this is a light stained zone (4) that includes a BR. The succeeding region up to the centromere is occupied by alternating dark and light bands. Centromeric region is composed of two heterochromatic blocks. The proximal end of arm D begins with an extra replicated zone followed by a light stained region interspersed with a small zone of dark bands. The distal end of arm D is flared up with the preceding region consisting of consecutive dark bands.

Chromosome 3 is acrocentric consisting of a very short arm and a very long arm constituting single complement (E). The free end of the short arm possesses a NOR. The short arm is very feeble with a constriction containing a dark band in it. The centromere has a heterochromatic block and the pericentromeric region of the short arm includes an extra-replicated region. Arm E consists of alternate dark and light bands throughout with flared distal end.

Chromosome 4 is also acrocentric consisting of arm F. The short arm of the chromosome bears a NOR. The proximal end of the arm is dark stained due to the presence of an extra replicated portion preceding the centromere. The region following the centromere consists of alternate thick and thin zones while the distal end has flared appearance.

The chromosome 5 is telocentric consists of arm G. The proximal end is round shaped containing an extra replicated portion following which there is a narrow constriction. The succeeding portion of the chromosome consists of alternate

thick and thin regions with constrictions in zone 4 and zone 6. The chromosome 6 is stumpy composed of arm H with an indistinct morphology. It is composed of an inconspicuous under replicated tiny arm with clear bands and an extra replicated stumpy long arm. The over-replication of this arm

is depicted by its enhanced width, approximately double the width of other longer chromosomes in the complement. The centromeric constriction is distinct in few complements. The under replication of the tiny arm is represented by its thin and feeble appearance.

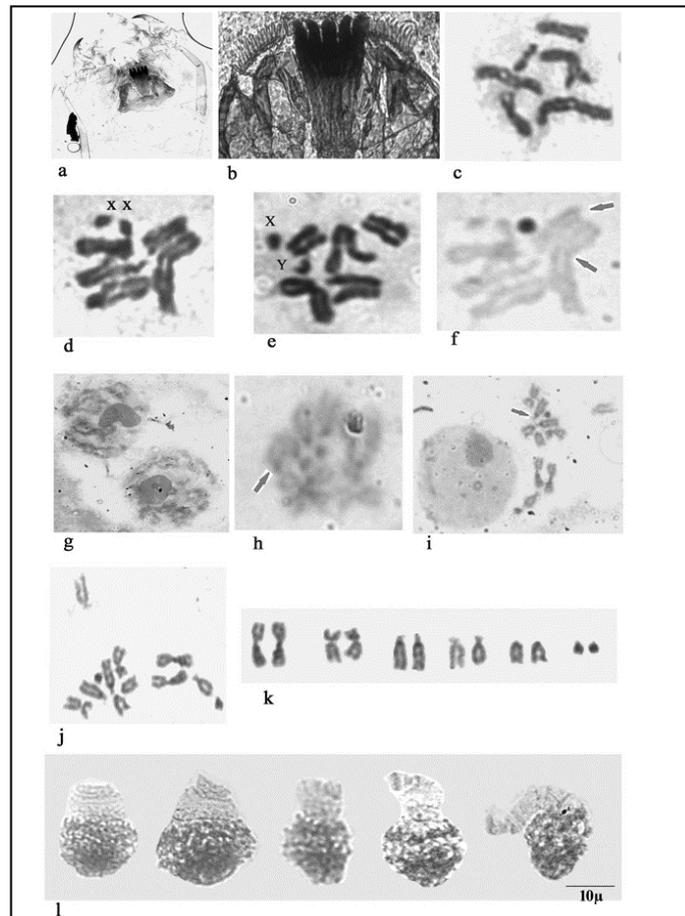


Fig 1: *Paramerina sp.*: a. Larval head showing Ligula paralogica, pecten hypopharynges, Antennae, Mentum and mandible under lower magnification; b. Enlarged view of Ligula, Paralogica and pecten hypopharynges; c. Meiotic prometaphase I showing terminal asynapsis and satellites; d. meiotic metaphase I of female; e. Meiotic metaphase of male; f. C-banded metaphase showing constitutive heterochromatin (arrows); g. Silver nitrate stained polytene complement showing NOR disposition; h, i. Silver nitrate stained somatic metaphase showing tererochromatin at centromeric regions; j. Geimsa stained somatic metaphase (2n=12); k. Karyotype of geimsa stained somatic metaphase; l. cutout of chromosome 6 from 5 different polytene nuclei

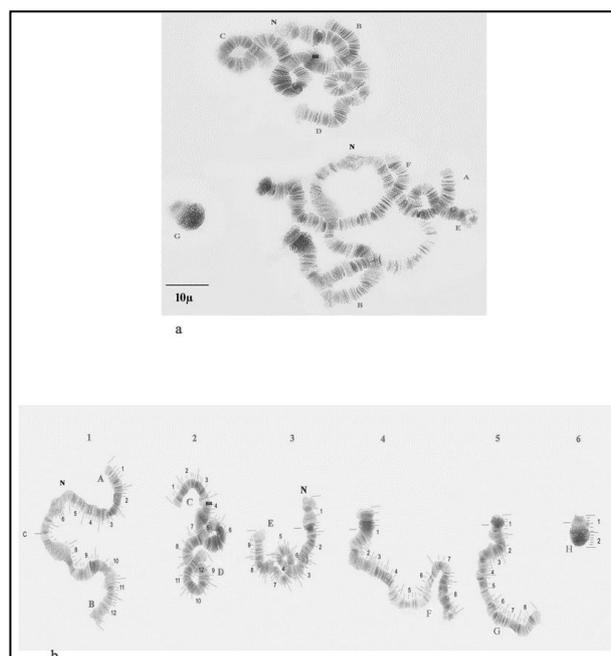


Fig 2: *Paramerina sp.*: a. LAO stained polytene chromosomal complement b. Cytophotomap of polytene chromosomes

Table 1: Image^J- Results -polytene chromosomes of *Paramerina sp.* (Measurements are in Microns)

Chromosome Pair	Length of Short arm (p)	Length of Long arm (q)	Total length (l)	arm ratio q/p	Centromere index (px100/l)	Relative length (lx100/L)	Morphology
1	10.77	14.14	24.9	1.31	43.25	24.9	Metacentric(m)
2	10.36	14.45	24.81	1.4	41.76	24.81	Metacentric(m)
3	2.5	17.27	19.76	6.91	12.65	19.76	Acrocentric (A)
4	2.45	13.69	16.14	5.58	15.18	16.14	Acrocentric (A)
5	1.11	11.14	12.25	10.04	9.06	12.25	Telocentric(t)
6	0.32	1.81	2.13	5.58	15.02	2.13	Acrocentric (A)

Table 2: Image^J- Results -Mitotic Metaphase chromosomes of *Paramerina sp.* (Measurements are in Microns)

Chromosome Pair	Length of short arm (p)	Length of long arm (q)	Total length (l)	arm ratio q/p	Centromere index (px100/l)	Relative length (lx100/L)	Morphology
1	11.89	12.68	24.57	1.07	48.39	24.57	Metacentric(m)
2	7.84	11.55	19.38	1.47	40.45	19.38	Metacentric(m)
3	3.2	16.09	19.29	5.03	16.59	19.29	Acrocentric (A)
4	3.64	12.33	15.97	3.39	22.79	15.97	Acrocentric (A)
5	0.81	11.86	12.67	14.55	7.02	12.67	Telocentric(t)
6	1.04	7.08	8.12	6.78	12.81	8.12	Acrocentric (A)

Discussion

The typical morphology of Ligula, Paraligula and Pecten epipharynges could be used in the genus level identifications. The morphological features in the larva such as the shape, length, number of teeth of Ligula, the number and type of projections in Paraligula, the number of teeth on the Pecten epipharynges have been of very characteristic in the genus and species level identifications. The available larval morphology features in the present study could be helpful upto genus level identifications. In the present study the species status could not be assigned to the material examined due to certain ambiguities of larval features, hence designated as unspecified species. The detailed morphology studies might be required to establish the species.

The banding pattern of polytene chromosomes of Chironomidae is well known to be useful in taxonomic and phylogenetic studies [20]. It has been proved that in the distinction of sympatric, closely related species the karyological studies have an advantage over morphological studies. The polytene chromosomes of each species are unique by having a set of specific features. They are an excellent model for studying chromosome structure, function and genomic organization of the species [42]. The karyotype of each species is described based on features, such as, diploid number of chromosomes (2n), the chromosome length ratio of each chromosomes in karyotype, position of centromere, number and position of nucleolous (N), position and size of Balbiani rings (BR) and puffs as well as the degree of conjugation of homologous chromosomes, polymorphism, presence of B chromosomes and of distinct sex chromosomes [27]. The Balbiani Rings (BRs) correspond to genes that code for proteins that are required to build larval tubes. The Nucleolar Organizer is an important cellular component required for synthesis of rRNA and the assembly of ribosomes. The ribosomes play a basic role in protein synthesis. The polytene characteristics observed of the studied material such as moderately sized balbiani rings, NORs, reasonably uniform polyteny are the exceptions of this material. Absence of polytene chromosomal polymorphism in the species proposes the ecological preferences of the larva that was observed during the collections. The populations collected were observed to be in 4-5 cm depth in the organic rich soil and also the collection sites were least polluted. This prompts the general phenomenon observed in Tanypodinae that they do not build their nests which require the proteins synthesized by them during larval life. The pattern of C-

bands is species specific and the organization of the constitutive heterochromatin can be used as a cytogenetic marker for species differentiation. The repetitive DNA that constitutes the component of heterochromatin evolves in different ways leading to its specificity giving clues to species differentiation.

The tiny pompon like chromosome noticed in our observations in the salivary complement of *Paramerina sp.* presence of which is characteristic of Tanypodinae seem to be similar to that observed in *Derotanypus sibiricus* [32], *Micropelopia paranebulosa* Fittkau [19], *Procladius choreus* Meigen [33, 34, 36], *Clinotanypus nervosus* Meigen [3, 36]. The morphology of pompon like chromosomes varies from much shortened to a flake like chromosome mass. The pompons probably represent sex chromosomes. Experiments with *Drosophila* show that most often pompons are formed from male X chromosomes seldom from female chromosomes or autosomes [43]. The importance of pompon-like chromosomes is not studied in Tanypodinae yet.

A unique situation of selective/differential polytenization was noticed by some authors in certain chironomids as was observed by Zacharias [40] in a Diamesian midge *Prodiamesa olivacea* a drastic reduction in the relative DNA content of the third chromosome in the mitotic chromosomes relative to the polytene chromosomes while maintaining the ratio relatively constant of other two chromosomes. A similar situation was noticed in another midge of subfamily Diamesia, *Pseudodiamesa branickii* the DNA content of tiny fourth chromosome in mitotic metaphase constituting 11% of the total DNA content reaches to 3.7% in polytenic state. Based on cytophotometry it was estimated that about 70 % of the DNA content was precluded during polytenic process. The prevalence of underreplication although was supported by Lakhotia [16, 15], Lakhotia and Sinha [14] and Zacharias [39] through their observations in some cases, some authors including Dennofer [8] and Lamb and Laird [17] had some reservations regarding this hypothesis. The highly heteromorphic nature of the tiny sixth chromosome that behaves differently in polytene somatic cells in the present study could imply that there might be a possible occurrence of differential endoreplication, as has been noticed in a few studies [2, 41, 40, 39, 28] in this regard. The variable expression and appearance of tiny short chromosome (figure 11) extra thicker than others in the complement in the present study pose to be a situation that could be resulted due to differential replication pertaining to the polytene complement. This reminds us the

observations by Mellad^[22] while describing the salivary gland chromosomes of *Anatopynia varius* (n=4) in which he noticed the fourth chromosome being very short and thicker than the remaining three elements. The observations made from the Indian material of *Anatopynia sp.*^[28], wherein, the chromosome 5 was found to have a conspicuous centromere differentiating an undereplicated inconspicuous short arm and an extra-replicated stumpy long arm with extrabound width more than the double the size of other chromosomes in the complement and about 5% more condensed in brain cells than in the follicular nuclei. These observations seem to add up to Mellad's findings.

Conclusions

Being the first attempt to present the polytene and mitotic metaphase chromosomal karyotypes of an unspecified species from the genus *Paramerina* (Diptera: Chironomidae), the present study has laid down a basis for further karyotypic descriptions of species in this genus in particular. Further studies are warranted to identification of the type material to the species level. The polytene, mitotic metaphase karyotypic descriptions and polytene chromosomal polymorphism studies of more species in the genus could help in the study of karyosystematics of species that are already described morphologically in the genus. To understand the nature of heterochromatin and the behavior of the short chromosome, further probing is required using cytophotometric and fluorescent staining methods.

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References

1. Ashe P, Cranston PS. Family Chironomidae. The Catalogue of Palaearctic Diptera. 1991; 2:113-499.
2. Bedo DG. Heterochromatin and disproportionate chromosome replication in *Anatopynia dyari* (Diptera: Chironomidae). Chromosoma. 1974; 48(2):181-90.
3. Belyanina SI, Sigareva LE. The karyotype of *Clinotanypus nervous* (Diptera, Chironomidae) from the Volga. Tsitologiya. 1981; 23(6):701-706.
4. Chaudhuri PK, Debnath RK. Two new species of *Paramerina* Fittkau (Diptera: Chironomidae) from India. Spixina Suppl. 1985; 11:167-171.
5. Chaudhuri PK, Hazra N, Alfred JR. A checklist of Chironomid midges (Diptera: Chironomidae) of the Indian subcontinent. Oriental Insects. 2001; 35(1):335-72.
6. Chowdaiah BN, Venkatachaliah G. Air-drying technique for the preparation of Mosquito chromosomes. The Nucleus. 1987; 30:44-46.
7. Cranston P. Biogeographic patterns in the evolution of Diptera. The evolutionary biology of flies. 2005, 274-311.
8. Denh ofer L. Underreplication during polytenization. Theoretical and Applied Genetics. 1982; 63(3):193-199.
9. Epler JH. Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. Special Publication SJ2001-SP13. 2001, 52.
10. Harrison AD. The non-biting midges (Diptera: Chironomidae) of South Africa. University of Waterloo, Canada. 2004.
11. Hazra N, Saha GK, Mazumdar A, Chaudhuri PK. Records of chironomids of the tribe Pentaneurini (Diptera: Chironomidae) in the Eastern Himalayas of India. In Annales de la Soci t  Entomologique de France, Taylor & Francis Group. 2011; 47(3-4):330-339.
12. Howell WT, Black DA. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Cellular and Molecular Life Sciences. 1980; 36(8):1014-1015.
13. Krosch MN, Cranston PS, Bryant LM, Strutt F, McCluen SR. Towards a dated molecular phylogeny of the Tanypodinae (Chironomidae, Diptera). Invertebrate Systematics. 2017; 31(3):302-316.
14. Lakhota SC, Sinha P. Replication in *Drosophila* chromosomes. Chromosoma. 1983; 88(4):265-276.
15. Lakhota SC, Tiwari PK. Replication in *Drosophila* chromosomes. Chromosoma. 1984; 89(3):212-217.
16. Lakhota SC. Independent endo-replication cycles of hetero and eu-chromatin in mitotic chromosomes in somatic cells of *Drosophila*. Proc Syrup" Cellular Control Mechanisms"(BARC, Trombay). 1982, 289-302.
17. Lamb MM, Laird CD. Three euchromatic DNA sequences under-replicated in polytene chromosomes of *Drosophila* are localized in constrictions and ectopic fibers. Chromosoma. 1987; 95(4):227-235.
18. Levan A, Fredga K, Sandberg AA. Nomenclature for centromeric position on chromosomes. Hereditas. 1964; 52(2):201-220.
19. Makarchenko EA, Petrova NA. Chironomids of Tanypodinae subfamily of Soviet Far East. I. Morphokaryological description of *Micropelopia paranebulosa* Fittkau. In: fauna, systematics and biology of freshwater invertebrates. Vladivostok 2. 1988, 8-35.
20. Martin J. Chromosomes as tools in taxonomy and phylogeny of Chironomidae (Diptera). Entomol. Scand. 1979; 10:67-74.
21. Martin J. The salivary gland chromosomes of *Chironomus oppositus* Walker (Diptera: Nematocera). Australian Journal of Zoology. 1969; 17(3):473-486.
22. Mellad AM. Types of Development of Polytene Chromosomes. Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences. 1942; 61(3):316-27.
23. Michailova PV. Polytene chromosomes and their significance to the systematics and phylogeny of the family Chironomidae, Diptera. Societas pro Fauna et Flora Fennica. 1989, 186-187.
24. Niitsuma H, Suzuki R, Kato H. Review of the Japanese species of *Paramerina* (Diptera: Chironomidae: Tanypodinae), with a key to the known males. Zootaxa. 2011; 2821(1):1-18.
25. Oliver DR, Dillon ME, Cranston PS. A catalog of Nearctic Chironomidae. Agriculture Canada. 1990, 89.
26. Paul N, Hazra N, Mazumdar A. Records of the Genus *Paramerina* (Diptera: Chironomidae: Tanypodinae) from Eastern Himalaya and Satpura Hill Regions of India. Neotropical entomology. 2013; 42(5):498-504.
27. Petrova NA, Zhirov SV. Characteristics of the Karyotypes of three subfamilies of chironomids (Diptera, Chironomidae: Tanypodinae, Diamesinae, Prodiamesinae) of the world fauna. Entomological review. 2014; 94(2):157-65.
28. Raju NG, Venkatachaliah G. Chromosomal Characteristics of an Indian Tanyponid Midge, *Anatopynia spp.* (Tanypodinae Chironomidae). Cytologia. 2004; 69(1):35-40.

29. Roback SS. The Tanypodinae (Diptera: Chironomidae) of Australia II. Proceedings of the Academy of Natural Sciences of Philadelphia. 1982; 134: 80-112.
30. Sæther OA. Family Chironomidae. Contributions to a Manual of Palaearctic Diptera (with special reference to flies of economic importance). 2000, 113-334.
31. Sasa M, Kikuchi M. Chironomidae (Diptera) of Japan. University of Tokyo Press. 1995, 333.
32. Sergeeva IV, Makarchenko EA. Morphokaryological Description of *Derotanypus sibiricus* (Kruglova et Tshernovskij, 1940) (Diptera: Chironomidae, Tanypodinae) from Siberia. Entomological science. 1999; 25;2(3):359-365.
33. Sergeeva IV. Karyotypes of Midges of the Subfamily Tanypodinae (Diptera, Chironomidae) of the World Fauna. Entomological Review. 1996a; 76(8):995-8.
34. Sergeeva IV. Revision of Tanypodinae (Diptera, Chironomidae) from the Volga River. I. Morphokaryotype analysis of *Procladius choreus*. Entomological Review. 1996; 75(5):65-74.
35. Sergeeva IV. Revision of tanypodinae from the Volga (Diptera, Chironomidae). 2. Morphological analysis of *Tanypus punctipennis*. Zoologicheskyy zhurnal. 1995b; 74(7):65-73.
36. Sergeeva IV. Specific Features in the Karyotypes of Chironomids of the Subfamily Tanypodinae (Diptera, Chironomidae); in Karyotaxonomy of Invertebrates: Collected Papers, Issue III, Ed. by V.E. Gokhman and V.G. Kuznetsova (Moscow). 1996b, 64-67.
37. Spies M, Reiss F. Catalog and bibliography of neotropical and Mexican Chironomidae (Insecta, Diptera). Spixiana Supplement. 1996; 22:61-119.
38. Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Experimental cell research. 1972; 75(1):304-306.
39. Zacharias H. Allocyclic behaviour and underreplication of the nucleolus chromosome in *Pseudodiamesa* (Chironomidae). Chromosoma. 1984; 89(4):263-273.
40. Zacharias H. Sex-linked difference in DNA content of a polytene chromosome in *Prodiamesa* (Chironomidae). Chromosoma. 1981; 82(5):657-672.
41. Zacharias H. Underreplication of a polytene chromosome arm in the chironomid *Prodiamesa olivacea*. Chromosoma. 1979; 72(1):23-51.
42. Zhimulev IF, Belyaeva ES, Semeshin VF, Koryakov DE, Demakov SA, Demakova OV, Pokholkova GV *et al.* Polytene chromosomes: 70 years of genetic research. International review of cytology. 2004; 241:203-275.
43. Zhimulev IF. Polytene chromosomes: morphology and structure. Science, Novosibirsk. 1992, 1-80.