

Morphological variation in the chromosome complement of *Neurobasis chinensis chinensis* of family Calopterygidae (Odonata: Zygoptera)

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Abstract: Chromosome complement of *Neurobasis chinensis chinensis* has been characterized by conventional staining and C-banding. The specimens were collected from Sadhupul (Solan), Ghumarwin (Bilaspur) of Himachal Pradesh and Lachhiwala (Dehradun) of Uttarakhand states of India. The species possesses $2n=23$ as the diploid chromosome number with XO-XX sex determination. Morphological variation in the chromosome complement has been observed in the species collected from different localities. In the spermatogonial metaphase, two large sized autosome pairs are present in specimens of Lachhiwala (Dehradun) and Sadhupul (Solan) and one large pair and other smaller than it are seen in specimens of Ghumarwin (Bilaspur). Similarly, in the diakinesis, two equal sized, large autosomal bivalents possess two interstitial and terminal chiasmata in the specimens of Lachhiwala (Dehradun) and Sadhupul (Solan). On the other hand, one extra large autosomal bivalent shows terminal and interstitial chiasmata, while second small bivalent reveals terminal chiasmata as remaining bivalents in the specimens of Ghumarwin (Bilaspur). These variations have also been confirmed by C-banding. In the diakinesis, C-bands are mostly present at the terminal ends of all the bivalents, while both the autosomal bivalents of Lachhiwala (Dehradun) and Sadhupul (Solan) specimens and one extra large autosomal bivalent of Ghumarwin (Bilaspur) specimens possess terminal as well as interstitial C-bands. X chromosome is C-positive throughout the length in all the specimens.

Keywords: Dragonfly; Odonata; Zygoptera; Calopterygidae; Morphological variation; C-banding; Conventional staining.

1. INTRODUCTION

Family Calopterygidae of suborder Zygoptera consists of 190 species organized into 21 genera worldwide, while 9 species under 6 genera are present in India. These damselflies are known as stream glories and are commonly found in hill streams. Among Odonata, these are also called as birds of paradise because of their beautiful metallic coloured wings. So far, 19 species of family Calopterygidae have been cytologically studied, among these, *Neurobasis chinensis chinensis* and *Vestalis apicalis* have been reported from India. Majority of the species possess $n=12$ (Oguma 1930, Makalowskaja 1940, Hirai 1956, Dasgupta 1957, Omura 1957, Kiauta 1967, 1968, 1970, 1971, 1972, 1975, Tyagi 1978, Kiauta and Kiauta 1982, Carnoy 1885, Walia and Sandhu 2002, Walia and Kaur 2011, Walia *et.al.* 2016). Taxonomically, 13 species of genus *Neurobasis* are present worldwide, while *Neurobasis chinensis chinensis* is the only species, which is available in India. Moreover, cytological data pertains to only *Neurobasis chinensis chinensis* species.

During the present study, variation in the chromosome complement has been studied in the *Neurobasis chinensis chinensis* collected from Sadhupul (Solan), Ghumarwin (Bilaspur) of Himachal Pradesh and Lachhiwala (Dehradun) of Uttarakhand states of India. Odonates have ambhibiotic life cycle, their larval forms are aquatic and adults are terrestrial. So, environmental conditions of the localities might be affecting the development of the larval forms, which is seen as morphological variation in the complement of species.

2. MATERIALS AND METHODS

Neurobasis chinensis chinensis damselfly has iridescent coloured body, broad head, conspicuous round eyes and broad rounded hindwings. Thorax is iridescent green and abdomen is longer than hindwings. Forewings are transparent with pale yellowish green tint, while hindwings are opaque and their basal two third parts are iridescent green or peacock blue in colour. On the other hand, females are dull in color and their wings are transparent, amber coloured with round creamy white opaque spot on the edges.

Live male specimens were collected from running water bodies and from plants twigs present near water bodies from localities Sadhupul (Solan), Ghumarwin (Bilaspur) of Himachal Pradesh and Lachhiwala (Dehradun) of Uttarakhand states of India during pre-monsoon and post-monsoon seasons of year 2015. Environmental conditions of these areas are different and summarized in Table-1. For the chromosomal studies, live specimens were dissected in 0.67% saline solution to remove the testes and were fixed in freshly prepared Carnoy's fixative (3:1, absolute alcohol: glacial acetic acid). After that, testes were teased on clean and dust free slides. Slides were air dried and stored in refrigerator for further investigations. Conventional staining has been done with carbol fuchsin stain by following the technique given by Carr & Walker (Carr and Walker, 1961) with slight modifications. C-banding has been done with giemsa stain by suggesting the technique given by Sumner (Sumner 1972) with minor modifications.

3. RESULTS

The chromosome complement of *Neurobasis chinensis chinensis* collected from different localities shows the morphological variation in the various stages of meiosis. In the spermatogonial metaphase plates of different localities, 23 elements are present, which include 22 autosomes and one X chromosome. Moreover, in the spermatogonial metaphase plates and karyotype, two large sized autosome pairs are seen in Lachhiwala (Dehradun) (Fig. 1a) and (Sadhupul) Solan specimens (Fig. 1b), while one large autosome pair and second pair, smaller than it are present in the Ghumarwin (Bilaspur) specimens (Fig. 1c). Metrical analysis of spermatogonial metaphase chromosomes of *Neurobasis chinensis chinensis* from different localities is given in the Table-2. In the diakinesis, 12 elements are visible. Among these, 11 are autosomal bivalents and one is oval shaped, X chromosome. Two equal sized, large autosomal bivalents with interstitial and terminal chiasmata are seen in the diakinesis plates of Lachhiwala (Dehradun) (Fig. 1d) and Sadhupul (Solan) specimens (Fig. 1e), while one extra large autosomal bivalent and second smaller than large bivalent are present in the Ghumarwin (Bilaspur) specimens (Fig. 1f). Remaining autosomal bivalents show single chiasma per bivalent. Similar morphological variations are also noticed in the metaphase-I complement of Lachhiwala (Dehradun) (Fig. 1g), Sadhupul (Solan) (Fig. 1h) and Ghumarwin (Bilaspur) (Fig. 1i) specimens, although, all the autosomal bivalents are rod shaped due to condensation and terminalization of chiasmata, while small X chromosome is mostly present at the periphery.

C- banding:

Morphological variations seen in the conventional staining have also been confirmed by C-banding. In the diakinesis, two large autosomal bivalents from Lachhiwala (Dehradun) (Fig. 1j) and Sadhupul (Solan) specimens (Fig. 1k) and one large autosomal bivalent from Ghumarwin (Bilaspur) specimens (Fig. 1l) possess terminal as well as interstitial C-bands, while remaining autosomal bivalents show terminal C-bands. X chromosome is C-positive for the entire length in all the specimens. Moreover, same variations are also seen in the complement and karyotype of metaphase-I plates of (Lachhiwala) Dehradun (Fig. 1m), (Sadhupul) Solan (Fig. 1n) and (Ghumarwin) Bilaspur (Fig. 1o) specimens, although C-bands are not clear due to the condensation of elements. Metrical analysis of metaphase-I chromosomes of *Neurobasis chinensis chinensis* from different localities has been done and is summarized in Tables-3, 4, 5. Number of diakinesis and metaphase-I stages obtained by conventional staining and C-banding in *Neurobasis chinensis chinensis* from different localities are shown in Table-6.

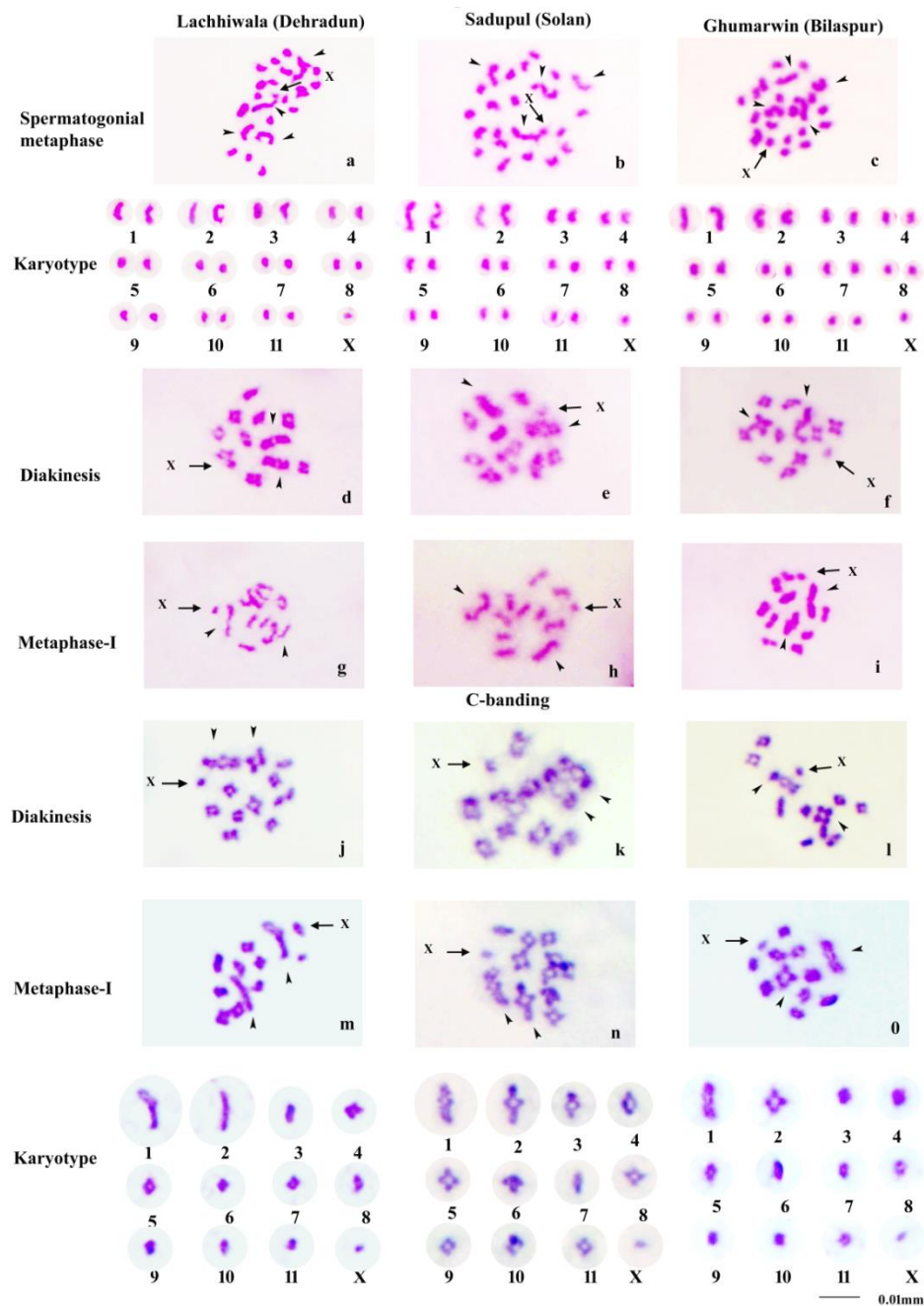


Fig 1: Explanation to figures (10X x 100X)

Normal complement and C-bands in *Neurobasis chinensis chinensis*. **Normal complement.** **1a.** spermatogonial metaphase and karyotype of Lachhiwala (Dehradun) specimens. **1b.** spermatogonial metaphase and karyotype of Sadhupul (Solan) specimens. **1c.** spermatogonial metaphase and karyotype of Ghumarwin (Bilaspur) specimens. **1d.** diakinesis of Lachhiwala (Dehradun) specimens. **1e.** diakinesis of Sadhupul (Solan) specimens. **1f.** diakinesis of Ghumarwin (Bilaspur) specimens. **1g.** metaphase-I of Lachhiwala (Dehradun) specimens. **1h.** metaphase-I of Sadhupul (Solan) specimens. **1i.** metaphase-I of Ghumarwin (Bilaspur) specimens. **C-bands.** **1j.** diakinesis of Lachhiwala (Dehradun) specimens **1k.** diakinesis of (Sadhupul) Solan specimens. **1l.** diakinesis of Ghumarwin (Bilaspur) specimens. **1m.** metaphase-I and karyotype of Lachhiwala (Dehradun) specimens **1n.** metaphase-I and karyotype of (Sadhupul) Solan specimens. **1o.** metaphase-I and karyotype of Ghumarwin (Bilaspur) specimens.

- **Bar** = 0.01 mm
- Largest autosomal bivalents are shown by arrow heads.

Table 1: Climatic conditions of different localities from *Neurobasis chinensis chinensis* specimens collected.

Sr. no.	Climate conditions	Lachhiwala (Dehradun)	Sadhupul (Solan)	Ghumarwin (Bilaspur)
1	Altitude	609 m a.s.l.	11,88 m a.s.l.	496 m a.s.l.
2	Temperature variation	1.3 ⁰ C in Jan to 34.7 ⁰ C in May	-4 ⁰ C in Jan to 32 ⁰ C in May	3 ⁰ C in December to 38.2 ⁰ C in May
3	Soil	Alluvial soil and non-calcic brown soil with valuable nutrients	Sandy loam and deficient in nutrients	Silty loam to silty clay with valuable nutrients

Table 2: Metrical analysis of Spermatogonial metaphase chromosomes of *Neurobasis chinensis chinensis* 2n (♂) = 23 (22A+X) from different localities.

Chromosome number	[Length in µm]		
	Lachhiwala (Dehradun)	Sadhupul (Solan)	Ghumarwin (Bilaspur)
1	7.69	5.38	6.92
2	7.69	5.38	6.92
3	7.32	5.38	5.38
4	7.32	5.38	5.38
5	3.84	3.84	3.07
6	3.84	3.84	3.07
7	3.07	3.07	3.07
8	3.07	3.07	3.07
9	3.07	2.30	3.07
10	3.07	2.30	3.07
11	3.07	2.30	3.07
12	3.07	2.30	3.07
13	2.30	2.30	3.07
14	2.30	2.30	3.07
15	2.30	2.30	2.30
16	2.30	2.30	2.30
17	2.30	2.30	2.30
18	2.30	2.30	2.30
19	2.30	2.30	2.30
20	2.30	2.30	2.30
21	2.30	2.30	2.30
22	2.30	2.30	2.30
23(Sexchromosome)	1.53	2.30	2.30

Table 3: Statistical analysis of Metaphase-I chromosomes of *Neurobasis chinensis chinensis* 2n=12 (11AA+X) from Lachiwala (Dehradun).

Chromosome number	Plate-1	Plate-2	Plate-3	Average	Average relative length
	[Length in µm]				
1.	9.790217	9.653813	9.432178	9.625403	0.177
2.	9.996035	9.554381	9.432185	9.660867	0.177
3.	4.646044	4.556541	4.712457	4.638347	0.085
4.	4.52501	4.53681	4.58634	4.549387	0.083
5.	4.082888	4.179215	4.142125	4.134743	0.076
6.	3.944612	3.941221	3.941682	3.942505	0.072
7.	3.66052	3.532145	3.516864	3.569843	0.065
8.	3.241211	3.425781	3.365456	3.344149	0.061
9.	3.241121	3.412453	3.193213	3.282292	0.06
10.	3.250371	3.249415	3.312141	3.270642	0.06
11.	2.538114	2.497902	2.512378	2.524567	0.046
12.	1.83026	1.812317	1.871553	1.838043	0.034
TCL	54.38079				

Table 4: Statistical analysis of Metaphase-I chromosomes of *Neurobasis chinensis chinensis* 2n=12 (11AA+X) from Sadhupul (Solun).

Chromosome number	Plate-1	Plate-2	Plate-3	Average	Average relative length
	[Length in μm]				
1.	9.58434	9.57457	9.497681	9.552197	0.146
2.	9.583334	9.568156	9.578197	9.576562	0.146
3.	5.839943	5.671456	5.791341	5.76758	0.088
4.	5.416667	5.415571	5.576541	5.469593	0.083
5.	5.418447	5.316785	5.417891	5.384374	0.082
6.	4.863095	4.768971	4.815698	4.815921	0.073
7.	4.722222	4.717187	4.723416	4.720942	0.072
8.	4.583333	4.641961	4.591651	4.605648	0.07
9.	4.498371	4.398761	4.317654	4.404929	0.067
10.	4.166667	4.234871	4.198761	4.2001	0.064
11.	4.168981	4.378191	4.231561	4.259578	0.065
12.	2.638889	2.717656	2.714567	2.690371	0.041
TCL	65.4478				

Table 5: Statistical analysis of Metaphase-I chromosomes of *Neurobasis chinensis chinensis* 2n=12 (11AA+X) from Ghumarwin (Bilaspur).

Chromosome number	Plate-1	Plate-2	Plate-3	Average	Average relative length
	[Length in μm]				
1.	8.775926	8.553471	8.432176	8.587191	0.177
2.	6.026832	5.987781	5.976325	5.996979	0.124
3.	4.391703	4.341266	4.234566	4.322512	0.089
4.	4.165884	4.015471	4.012234	4.06453	0.084
5.	3.993428	3.663956	3.721342	3.792909	0.078
6.	3.974591	3.875646	3.875481	3.908573	0.08
7.	3.584938	3.279567	3.121342	3.328616	0.068
8.	3.427074	3.426167	3.165386	3.339542	0.069
9.	3.013416	3.218675	3.132134	3.121408	0.064
10.	3.016527	3.014356	3.341673	3.124185	0.064
11.	2.876442	2.674545	2.567458	2.706148	0.055
12.	2.195851	1.932176	2.067865	2.065297	0.042
TCL	48.35789				

Table 6: Number of Diakinesis/Metaphase-I plates studied in *Neurobasis chinensis chinensis* collected from different localities:

Locality	Conventional staining		C-banding	
	Diakinesis	Metaphase-I	Diakinesis	Metaphase-I
Ghumarwin (Bilaspur)	15	10	7	20
Solan (Sadhupul)	6	7	9	8
Dehradun (Lachhiwala)	10	12	20	8
Total	31	29	36	36

4. DISCUSSION

Type number of the family Calopterygidae is n=12. So far, 19 species of family Calopterygidae have been cytologically studied, among these, 2 species are from India. Majority of the species possess n=12 (Oguma 1930, Makalowskaja 1940, Hirai 1956, Dasgupta 1957, Omura 1957, Kiauta 1967, 1968, 1970, 1971, 1972, 1975, Tyagi 1978, Kiauta and Kiauta 1982, Carnoy 1885, Walia and Sandhu 2002, Walia and Kaur 2011, Walia *et.al.* 2016) while few variations in the chromosome complement have also been reported. Kiauta (1968) observed variation in size, origin and taxonomic value of m chromosomes in *Calopteryx virgo*. Later, Kiauta (1971) noticed the chromosomal fragmentation of medium sized bivalent and precocious segregation of one bivalent in *Calopteryx virgo meridionalis* (n=13, 14) from northwestern Spain. He also stated that population of the species was highly unstable, cytogenetically.

Taxonomically, 13 species of genus *Neurobasis* are available worldwide, while *Neurobasis chinensis chinensis* is the only species, which is available in India. Moreover, cytological data pertains to only *Neurobasis chinensis chinensis* species. Variation in karyotype of *Neurobasis chinensis chinensis* has been reported by many researchers. Kiauta (1975) noticed two complements, one $n=13$ and other $n=12$ (resulted due to the fusion of two autosomes) from Nepal. Later, Tyagi (1978) reported $n=12$ from Dehradun valley; Kiauta and Kiauta (1982) observed $n=12m$ from Arun valley, Eastern Nepal; Walia and Sandhu [2002] recorded $n=12m$ from Himachal Pradesh. Recently, Walia *et al.* (2016) reported $n=12$, without m bivalent from Andretta (Kangra) of Himachal Pradesh. During the present study, $n=12$, without m bivalent has been observed in the same species from different localities and results are in accordance to the reports given by Tyagi (1978) and Walia *et al.* (2016). Moreover, morphological variation in chromosome complement has been recorded in the specimens of different localities. Two equal sized large autosomal bivalents are present in the specimens of Lachhiwala (Dehradun) and Sadhupul (Solan), while one extra large and second small bivalent are seen in the specimens of Ghumarwin (Bilaspur). Results of the species from Ghumarwin (Bilaspur) of Himachal Pradesh are in accordance to the results reported on the same species from Andretta (Kangra) of Himachal Pradesh by Walia *et al.* (2016). These results are also confirmed by the C-banding. In Odonata, fragmentations and fusions of chromosomes are very common due to the presence of holocentric chromosomes. These types of variations in complement favor the adaptability of the species in the different environmental conditions. Presently studied *Neurobasis chinensis chinensis* species is cytogenetically unstable as the variation in complement ($n=13$, $n=12$, $n=12m$) has been earlier reported by various authors. During the present study, variation in the size of chromosomes in the complement might be due to the climatic conditions as odonates have ambhibiotic life cycle, their larval forms are aquatic and adults are terrestrial. So, the environmental conditions of the localities might be affecting the development of the larval forms, which is seen in the form of morphological variation in the complement of species.

5. CONCLUSION

Morphological variation in the chromosome complement of *Neurobasis chinensis chinensis* collected from different localities has been observed and has also been confirmed by conventional staining and C-banding. Variation in the size of chromosomes in the complement might be due to the climatic conditions as odonates have ambhibiotic life cycle, which might be affecting the development of the larval forms.

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