Cytological Studies in Three Species of *Tolypella*

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The genus *Tolypella* Leonh. is among one of the rare members of Charophyta. 12 taxa belonging to this genus have been studied so far from different corners of the world (cf. Lindenbein 1927, Corillion 1960, Hotchkiss, 1963, Sawa 1974 and Guerlesquin 1965, 1967) but there appears to be no contribution on its karyology from India except Sarma and Ramjee (1969) who have worked out the cytology of only one taxon viz. *T. intricata* f. *prolifera*. In the present investigation three taxa belonging to this genus viz. *T. nidifica*, *T. nidifica* var. *nidifica* f. *stenhammariana* and *T. jwalai* (sp. nov.) have been cytologically investigated.

Material and methods

Three taxa under study were collected from 2 districts of North Bihar viz. Gopalganj and West Champaran, details of which may be seen in the Table 1.

Table 1. Details of collection

S1. no.	Name of the taxa	Date of collection	Place of collection	Habitat
1.	<i>Tolypella</i> Leonh. <i>nidifica</i> (O. Mill) A. Br., em.	February 1976	Bansibatranha, Hathwa, Gopalganj	Paddy field
2.	<i>Tolypella nidifica</i> var. <i>nidifica</i> f. <i>stenhammariana</i> (Walm in lilj) R.D.W.	February 1979	Kumarbag, West Champaran	Paddy field
3.	<i>Tolypella jwalai</i> sp. nov.	February 1976	Mishra Batranha, Hathwa, Gopalganj	Paddy field

Cytological preparations were made from the antheridial filaments. Fixations of the healthy branches carrying sex organs were made on the collection spots between 8-10 A.M.

Carnoy's acetic-alcohol (1 : 3) was used as the fixative and iron-alum aceto-carmine method (cf. Godwards 1948) was adopted throughout the study for cytological preparations.

The characteristics of the karyotype were worked out after ascertaining:

- i) Length of the metaphase chromosomes.
- ii) Total chromatin length as ascertained at metaphase.
- iii) TF% as per Huziwara (1962) i.e.

$$TF\% = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}} \times 100$$

iv) Coefficient of variation (C.V.) as per following equation:

$$C.V. = \frac{\text{Standard deviation}}{\text{Mean length of chromosome}} \times 100$$

Cytological characters like chromosome number, TF%, coefficient of variation and total chromatin length were taken as the parameters for computing relative distances between the pairs of taxa at hand.

Relative distances between the taxa were computed through "Ordinary Euclidean" distance method (cf. Duran and Odell 1974). The equation of which may be read as follows:

$$d_{ij} = \left[\sum_{K=1}^P (X_{iK} - X_{jK})^2 \right]^{1/2}$$

where $i(j)$ = taxon (1, 2, 3 ... and n)

d_{ij} = distance between i th and j th taxa

P = the number of cytological characters to be compared and

X_{iK} = value of K th characters on i th taxa

The cluster analysis was performed in accordance with the Tocher's method (cf. Rao 1952).

The average within cluster distances were obtained by dividing the total within distance by the total number of pairs for each cluster thus formed. The average inter cluster distances among different pairs of taxa by the product of number of taxa in any pair of clusters.

Cytological observation

Karyological observation on three taxa viz. *T. nidifica*, *T. nidifica* var. *nidifica* f. *stenhammariana* and *T. jwalai* has revealed remarkable similarities as regards the organisation of nuclei and events of mitoses being on the pattern of other genera like *Chara* and *Nitella*. However, the overlapping of chromosomes at metaphase has been noted as a common feature in all the three taxa at hand which made correct counting of the chromosomes difficult.

Interphase nuclei, single nucleolus, which was constantly observed as dense spherical structure within the former and metaphase chromosomes have been carefully measured (cf. Table 2).

A count of 15, 11 and 6 chromosomes was ascertained at metaphase for *T. nidifica* (Fig. 1), *T. nidifica* var. *nidifica* f. *stenhammariana* (Fig. 2) and *T. jwalai* (Figs. 3 and 4) respectively. The length of two arms with respect to centromere has been measured and from this idiograms have been drawn (Figs. 5-7).

The total chromatin length, TF% and coefficient of variation as computed for the karyotypes of three taxa under study have been summarised in the Table 3 along with respective chromosome number.

Cluster analysis by distance suggests inclusion of *T. nidifica* and *T. nidifica* var. *nidifica* f. *stenhammariana* in one cluster having intra-cluster distance of 21.935 and *T. jwalai* in a separate cluster with inter-cluster distance of 129.011 (Fig. 8).

Figs. 1-4. 1, *T. nidifica* (O. Mill.) A. Br. em., R.D.W. $n=15$. $\times 3200$. 2, *T. nidifica* var. *nidifica* f. *stenhammariana* (Wall. in Lilj.) R.D.W. three metaphase plates with $n=11$. $\times 3900$. 3 and 4, *T. jwalai* sp. nov. showing $n=6$ at metaphase and at anaphase. $\times 3700$ respectively.

Table 2. Details of nuclei, nucleolus and range of chromosome length

Sl. no.	Name of the taxa	Diameter of nuclei (μm)	Diameter of nucleoli (μm)	Range of chromosome length (μm)
1.	<i>T. nidifica</i>	5.5-11.0	1.76-2.2	3.3-11.0
2.	<i>T. nidifica</i> var. <i>nidifica</i> f. <i>stenhammariana</i>	6.6-11.0	1.76-2.64	4.4-11.0
3.	<i>T. jwalai</i>	4.4-11.0	2.2	2.2-6.6

Discussion

Variations in the diameter of nuclei and that of nucleoli among three taxa at hand have been recorded within the range merging in one another (cf. Table 2) and hence are of least taxonomic importance. Overlapping of long metaphase chromo-

Idiograms 5-7. 5, *T. nidifica* (O. Müll.) A. Br. em., R.D.W. 6, *T. nidifica* var. *nidifica* f. *stenhammariana* (Wall. in Lilj.). R.D.W. 7, *T. jwalai* sp. nov.

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Fig. 8. Diagrammatic representation of average inter and intra cluster distances between investigated three taxa of *Tolypella* Leonh. (Roman numbers: clusters number; Hindu numbers: intracluster distance). Cluster-I includes 1 and 2 and Cluster-II *T. jwalai*.

Table 3. Details of total chromatin length, TF% and coefficient of variation

S1. No.	Name of the taxa	Number of chromosome (n)	Total chromatin length (μ m)	TF %	Coefficient of variation
1.	<i>T. nidifica</i>	15	105.7	39.5	27.3
2.	<i>T. nidifica</i> var. <i>nidifica</i> f. <i>stenhammariana</i>	11	85.6	40.0	35.1
3.	<i>T. jwalai</i>	6	31.9	34.4	30.5

somes observed by the authors is consistent with the report of Hotchkiss (1963, 1966).

The number of chromosomes has been ascertained as $n=15$ for *T. nidifica*, $n=11$ for *T. nidifica* var. *nidifica* f. *stenhammariana* and $n=6$ for *T. jwalai*. From the available literature, it appears that the chromosome number in *T. nidifica* var. *nidifica* f. *stenhammariana* ($n=11$) and *T. jwalai* ($n=6$) has not been reported so far and so they are new to the science. Present count in *T. nidifica* ($n=15$) is at variance with the report of Lindenbein (1927) who has reported $n=42$ for the same species.

The chromosome number reported so far in the taxa other than those worked out in the present study, is highly variable, ranging between 8 to 50 (cf. Table 4).

Table 4. Chromosome number reported so far in the taxa belonging to the genus *Tolypella* other than those investigated by the authors

Sl. No.	Name of the taxa	Chromosome number (n)	Worker's name and year
1.	<i>T. hispanica</i> Nordst.	10	Corillion and Guerlesquin 1959
2.	<i>T. hispanica</i> var. <i>Poteri</i> Daily	11	Hotchkiss 1966
3.	<i>T. glomerata</i> (Desv. in Lois) Leonh.	15 ca 20 33	Corillion and Guerlesquin 1959 Guerlesquin 1964, 1967 Sawa 1974
4.	<i>T. comosa</i> T.F.A.	33	Sawa 1974
5.	<i>T. nidifica</i> (O. Mill) A. Br. Sub. Sp. <i>Occidentalis</i> R. Cor.	ca 42 ca 25	Lindenbein 1927 Guerlesquin 1964
6.	<i>T. salina</i> R. Cor.	50	Corillion 1960
7.	<i>T. prolifera</i> (Ziz. ex. A. Br. Leonh.)	10 11	Guerlesquin 1961, 1967 Hotchkiss 1966
8.	<i>T. intricata</i> (Trent. ex. Roth) Leonh.	11	Sawa 1974
9.	<i>T. intertexta</i> (Trent. ex. Roth) Leonh.	11	Hotchkiss 1966
10.	<i>T. canadensis</i> Sawa	8	Sawa 1974
11.	<i>T. boldii</i> Sawa	8	Sawa 1974
12.	<i>T. intricata</i> var. <i>intricata</i> f. <i>prolifera</i> (Ziz. ex. A. Br) R.D.W.	11	Sarma 1968, Sarma and Ramjee 1971

Perusal of the chromosome counts in the Table 4 dose not permit consideration of any simple polyploid series in the genus. Probably due to this, there has been a controversy over the basic number of the genus *Tolypella*. While Hotchkiss (1966) has proposed 11 as the basic number of this genus, Guerlesquin (1961, 1967) has suggested the basic numbers 5 or 10 for its European species. Report of $n=33$ in two taxa (cf. Table 4) goes in favour of considering 11 as the basic number but the discovery of two lower counts viz. $n=6$ (present study) and $n=8$ (Sawa 1974) come in its way. However, a long polyploid series (10, 15, 20, 25 and 50) in multiple of 5 may be visualised within the genus. In addition, two more possible polyploid series viz. $n=6$ and 42 and $n=11$ and 33 may also be seen. Therefore, if any effort is made to consider 5 and 11 as the basic number of the genus *Tolypella*, 6 and other

unrelated count i.e. 8 deserve the same consideration. Sawa (1974) appears to be justified in deriving the different counts from the ancestral genome of 3-chromosome complement through aneuploidy at different levels of ploidy. Hotchkiss (1966) has also supported the derivation of $n=11$ from $n=12$ through the loss of one chromosome. Present count of $n=6$ in *T. jwalai* supports the existence of ancestral genome consisting of three chromosomes proposed by Sawa (1974). Different short polyploid series in the genus may be considered as an attempt towards establishment of distinct polyploid series in the tribe Chareae and Nitelleae.

The length of the chromosomes among three investigated taxa has been recorded between 2.2 μm and 11.0 μm . There appears to be no previous record on the length of the chromosomes in this genus except Ramjee (1968) who has reported the range of chromosome length from 1.7 to 3.3 μm in *T. nidifica* f. *prolifera*.

Karyotype analysis in three species has revealed that in addition to chromosome number, they are distinct as regard the total chromatin length, TF% and coefficient of variation among the chromosome complements (cf. Table 3). However, the aforesaid data do not throw any light on their phylogenetic relationship.

Adhering to the view of progressive evolution in Charophyta along with increase in the number of chromosomes (cf. Sarma *et al.* 1970 and Sawa 1973), *T. jwalai* possessing the lowest count ($n=6$) should be regarded as the most primitive and the rest two (having $n=11$ and $n=15$) as comparatively advanced. But *T. jwalai* with lowest total chromatin length and TF% may be regarded as advanced than *T. nidifica* and *T. nidifica* var. *nidifica* f. *stenhammariana*. Even on the basis of coefficient of variation, *T. jwalai* keeps an intermediate position between the two. Similarly, *T. nidifica* var. *nidifica* f. *stenhammariana* with $n=11$ should be regarded as the primitive than *T. nidifica* with $n=15$. But comparatively lower total chromatin length and higher coefficient of variation in the former points to its advancement over the latter. The sequential phylogenetic relationship can be expected only when there is unidirectional evolution. Unrelated features of primitiveness and advancement with different counts i.e. 6, 11 and 15 speak of their derivation along three independent lines of chromosomal differentiation in different time at different ploidy level.

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Summary

Three species of *Tolypella* Leonh. viz. 1, 2 and 3 have been cytologically investigated and their karyotypes have been worked out. The chromosome numbers in last two named taxa have been reported for the first time and hence are new to the science. In *T. nidifica*, present count is at variance with the previous report. Taxonomical distinction of taxa at hand has been discussed in the light of conclusions

drawn from cluster analysis (by distance) on the basis of karyotypic data like chromosome number, total chromatin length, TF% and coefficient of variation.

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