

Research Article

A karyotype study in two fish species belonging to Genus *Neolissochilus* found in Meghalaya, India

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Abstract

The karyomorphological study of two species of Mahseer belonging to the genus *Neolissochilus*, namely *Neolissochilus hexagonolepis* and *N. hexastichus* were carried out. The study revealed the basic chromosome number in both the Mahseer species was observed to be 100. However, the karyotype formula number varied among the species. *N. hexagonolepis* had a diploid chromosome number of 42 metacentric (m), 20 submetacentric (sm), 8 subtelocentric (st) and 30 telocentric (t) and *N. hexastichus* had a karyotypic formula of 32 metacentric (m), 22 submetacentric (sm), 4 subtelocentric (st) and 42 telocentric (t). This finding removed taxonomic confusion due to the differences in the chromosome number, the morphology of the chromosomes and chromosome formula between the two fish species of the genus and helped in distinctive and unblemished identification of the two species belonging to the genus *Neolissochilus* from Meghalaya, though they have a morphological similarity.

Keywords: Mahseer, *Neolissochilus hexagonolepis*, *Neolissochilus hexastichus*, Karyotype, Taxonomic

INTRODUCTION

Cyprinid fishes of the genus *Neolissochilus* are naturally found throughout tropical and subtropical areas of southern and south-eastern Asia (Rainboth, 1885). The importance of these mahseers is due to their delicacy, high protein content (Day, 1876) as food and as game fish. The fishes in angling tourism are gaining importance. Several organisations, through the development of sanctuaries, are working for their conservations (Joshi *et al.*, 2018) in Meghalaya, in particular, such as establishing 54 sanctuaries till date since 2012 in Garo, Khasi and Jaintia Hills Districts (Dash *et al.*, 2020). *Neolissochilus hexagonolepis* and *N. hexastichus* are cyprinids found in the rivers of Meghalaya, India. The Chocolate mahseer or *N. hexagonolepis*, one of the important fish species, is a highly esteemed food and game fish found in the North-Eastern Himalayan region, particularly in Meghalaya. The fish is considered a threatened species and needs special attention to conserve to increase its population in natural water bodies (International Union for Conservation of Nature, 2021). Three different water bodies, viz., River Khri of Umiam,

East Khasi Hills, River Umran and River Umraleng of Ri Bhoi District, Meghalaya, India were explored for the presence of mahseer species and studies on the water parameters of these rivers were found to be conducive for mahseer growth and propagation (Sarma and Bhuyan, 2007).

N. hexastichus, on the other hand, is found only in the Janiaw river of Mawsynram in East Khasi Hills District of Meghalaya during our survey and is a confusing species due to its similarities to *Tor tor* (Menon, 1974). Brown mahseer or *N. hexastichus* has been claimed as a valid species which has characteristics different from other species of *Tor* as the *Tor* characters in this species are very poorly represented (Day, 1878) and resurrected from synonymy *Tor tor*. *N. hexastichus* is survived with a small population in the river Diyung in Dima Hasao district in Assam, which is perhaps its last stronghold (Laskar, 2013; Kar and Khyntiam, 2020). Hence, a thorough survey on the occurrence of this species in another locality of rivers of Meghalaya, India, is urgent.

A prominent study by Sen and Jayaram (1982) concluded that *N. hexastichus* can not be included under

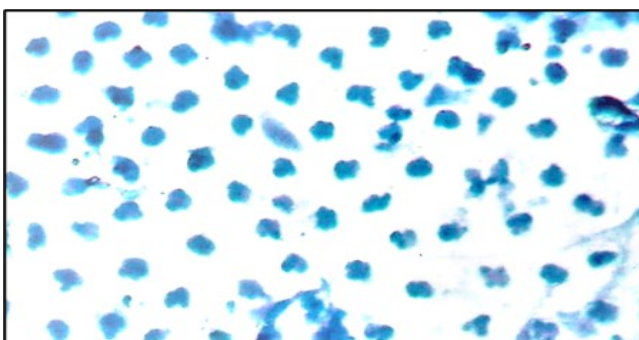
Tor genus and lips are thick with a continuous labial groove in *Tor* and an interrupted groove in *Neolissochilus*. Among the mahseer cyprinids, an easily distinguishable character is relatively large on their body (Desai, 2003) and *Tor* can be distinguished from other cyprinids by having a fleshy median mental lobe (Roberts, 1999). Due to the similarities in the morphometric characters and meristic counts, difficulties have been encountered in the correct identification of the mahseer belonging to *Neolissochilus*. This group of fishes and chromosome karyotype study and molecular techniques are required to resolve such ambiguities.

One of the important strategies for the conservation of fish is the artificial propagation of the species, either in-situ or ex-situ, which helps run the rivers or other natural habitats. However, the first step for breeding programmes is the correct identification of a fish species with proper taxonomical tools. The most reliable taxonomic information should be collected from literature and extensive examination of specimens and taxonomic and systematics studies have a high standard from the rest of science (Vecchione *et al.*, 2000). For evolutionary status, the main source of information is the morphology of the fish specimens. For evolutionary status, the main source of information is the morphology of the fish specimen.

The present study will provide a better insight into the presence of the particular fish species *N. hexastichus* in Meghalaya, India, and its differences from *N. hexagonolepis*, an abundant fish species found in many rivers of Meghalaya.

MATERIALS AND METHODS

The sampling of fishes (*N. hexagonolepis* and *N. hexastichus*) was done from two places Janiaw River of East Khasi Hills District and Umlaleng River of Jaintia Hills District of Meghalaya. A total of 6 fishes were used for karyotype since only a limited number of *N. hexastichus* species were obtained. The fishes were brought to the Fishery Science Department of St. Anthony's College and acclimatized in ponds and no Animal ethics approval is required for the capture of fishes since the healthy ones are kept for breeding programs.



Morphometric characters and Meristic counts were employed to confirm the correct identification of fish species with the help of measuring length, weight, counting of fins and many specific characters (Cavalcanti *et al.*, 1999) and the specific keys used are paper-based. The species were further supported by ZSI (Zoological Survey of India), Shillong, in which the samples from the Janiaw river and Umlaleng river were sent for species confirmation.

The study of chromosomes through cytogenetic spreads was conducted in the two species of *Neolissochilus*. For Karyotypic studies, healthy fishes were considered and each fish was injected intramuscularly with 0.01 per cent Colchicine at a dose of 1ml/100g bodyweight of the fish. The fishes were kept in a well-aerated aquarium for 2 hours. The fishes were then anaesthetized using chloroform for 5 minutes. The gills and kidney tissues were isolated and immediately, the tissues were processed following the KCl-Acetomethanol-flame drying method (Barat *et al.*, 2012). The slides were then stained with 5 per cent Giemsa stain (pH 6.8) for 2 hours, washed in distilled water, mounted in DPX (Dibutylphthalate Polystyrene Xylene). The slides were observed under Trinocular Microscope with immersion objective and images were taken using a Motic camera. The karyomorphological identification was made based on the length of the p arm, length of the q arm, arm ratio (length of the long arm to the short arm of the chromosome) calculated and separated as metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t) as per Levan *et al.* (1964).

RESULTS AND DISCUSSION

The cytogenetic spread of the light micrograph of both species is shown in Fig. 1. The chromosome number was found to be 100, which was consistent with the chromosome number in carps. During karyomorphological care was taken to measure only the visible and -defined chromosome arms. Table 1 and Table 2 represent the calculated p arm, q arm, arm ratio, centromeric index of *N. hexagonolepis* and *N. hexastichus*, respectively, based on which the chromosome type was then categorised .

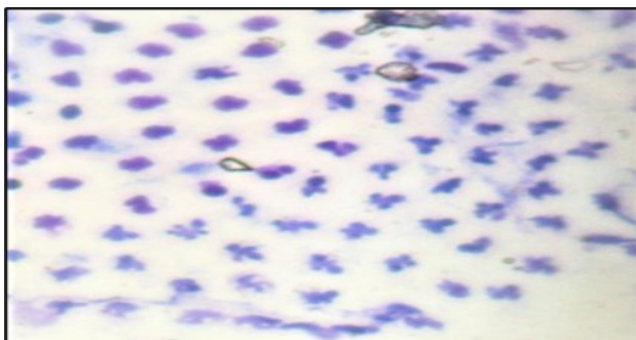


Fig.1. Light micrograph showing Cytogenetic spread

This study is the first reported from the region and if available, the reports are scanty if at all available. It was found that the chromosome number in both the species was 100 and it was concluded that the number of chromosomes is conserved as seen in mahseer species of cyprinids. Since the chromosome size is small and usually abundant and has more contracted structures, studying and measuring fish chromosomes is somewhat more difficult than mammals (Suleyman *et al.*, 2004; Saxena and Vasave, 2012). The most commonly occurring diploid number in family Cyprinidae is 50, considered to be the modal number in the case of this family (Manna, 1983; 1984; Rishi, 1989) and also seen in the family *Puntius* (Ganai and Yousuf, 2011),

which is valid over 80 per cent of metaphase spread. Sahoo *et al.* (2007) showed a karyotype of 32 metacentric (m), 16 sub-metacentric (sm), 6 sub-telocentric (st) and 46 telocentric (t) on the karyotype analysis of *N. hexagonolepis*. However, the present study recorded 42 metacentric (m), 20 submetacentric (sm), 8 sub-telocentric (st) and 30 telocentric (t) for *N. hexagonolepis* as shown in Table 1 and Figure 2, whereas for *N. hexastichus* it was 32 metacentric (m), 22 submetacentric (sm), 4 subtelocentric (st), 42 telocentric (t) as shown in Table 2 and Figure 3. The presence of similar chromosome number ($2n=100$) with varied karyotypes in Mahseer species of *Neolissochilus* and *Tor* species suggests the evolution among both the species through

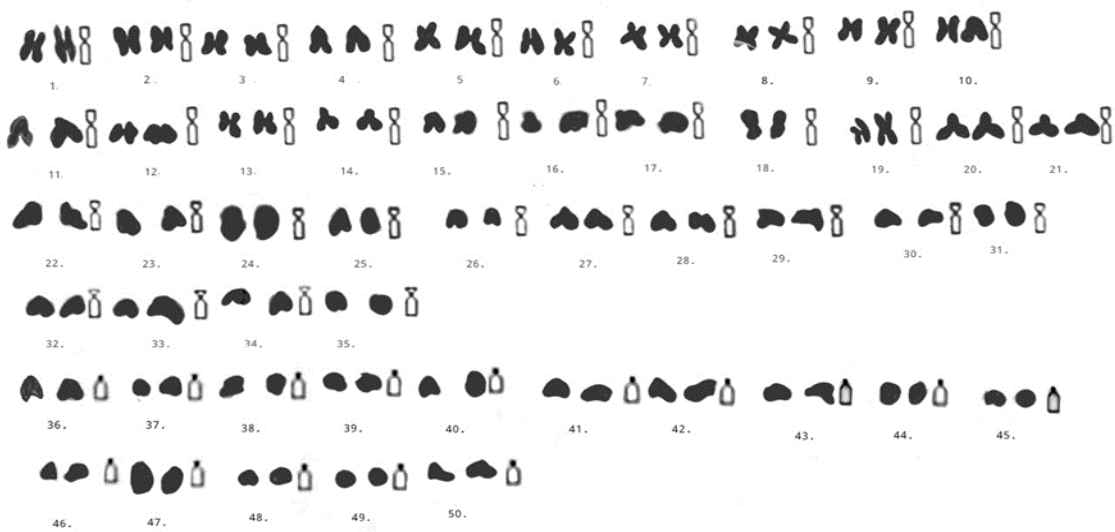


Fig. 2. Arrangements of chromosomes of *N. hexagonolepis* in pairs in according to their morphological appearances.

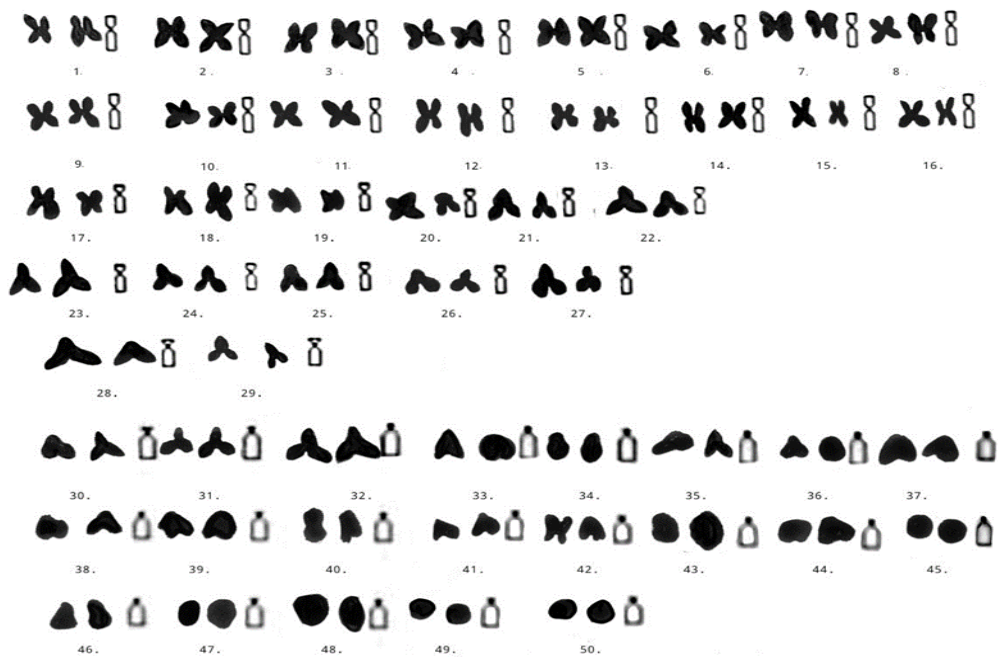


Fig. 3. Arrangements of chromosomes of *N. hexastichus* in pairs in according to their morphological appearances

Table 1. Karyomorphology of *N. hexagonolepis* chromosomes.

S.No	p arm (µm)	q arm (µm)	Arm ratio	Total length (µm)	Centeromeric index	Chromosome type
1	1.8	1.35	1.25	2.43	44.44444444	M
2	1.21	1.12	1.080357143	2.33	48.06866953	M
3	1.44	1.36	1.058823529	2.8	48.57142857	M
4	1.57	1.57	1	3.14	50	M
5	0.7	0.5	1.4	1.2	41.66666667	M
6	0.5	0.7	1.4	1.2	41.66666667	M
7	0.5	0.6	1.2	1.1	45.45454545	M
8	0.3	0.4	1.333333333	0.7	42.85714286	M
9	0.6	0.6	1	1.2	50	M
10	0.6	0.8	1.333333333	1.4	42.85714286	M
11	0.6	0.5	1.2	1.1	45.45454545	M
12	0.4	0.8	2	1.2	33.33333333	SM
13	1.1	1.0	1.1	2.1	47.61904762	M
14	0.8	0.6	1.333333333	1.4	42.85714286	M
15	0.7	1.0	1.428571429	1.7	41.17647059	M
16	0.4	0.7	1.75	1.1	36.36363636	SM
17	0.7	0.7	1	1.4	50	M
18	0.9	0.8	0.888888889	1.7	41.17647059	M
19	0.5	1.0	2	1.5	60	SM
20	0.6	0.4	1.5	1	40	M
21	0.4	0.9	2.25	1.3	30.76923077	SM
22	0.6	0.9	1.5	1.5	40	M
23	0.4	0.5	1.25	0.9	44.44444444	M
24	0.6	0.6	1	1.2	50	M
25	0.7	0.9	1.285714286	1.6	43.75	M
26	0.6	0.7	1.166666667	1.3	46.15384615	M
27	0.6	0.6	1	1.2	50	M
28	0.6	0.4	1.5	1	40	M
29	0.7	0.5	1.4	1.2	41.66667	M
30	0.4	0.7	1.75	1.1	36.36363636	SM
31	0.2	0.8	2	1	20	SM
32	0.5	0.6	1.333333333	1.1	45.45454545	M
33	0.5	0.6	1.333333333	1.1	45.45454545	M
34	0.7	0.6	1.166666667	1.3	46.15384615	M
35	0.8	0.8	1	1.6	50	M
36	0.8	0.8	1	1.6	50	M
37	0.7	0.2	3.5	0.9	22.22222222	ST
38	0.8	0.9	1.125	1.7	47.05882353	M
39	0.6	0.7	1.166666667	1.3	46.15384615	M
40	0.5	0.4	1.25	0.9	44.44444444	M
41	1.0	0.5	2	1.5	33.33333333	SM
42	0.7	0.5	1.4	1.2	41.66666667	M
43	1.4	0.5	2.8	1.9	26.31578947	SM
44	0.6	0.7	1.166666667	1.3	46.15384615	M
45	0.9	0.4	2.25	1.3	30.76923077	SM
46	1.1	0.4	2.75	1.5	26.66666667	SM
47	1.4	0.2	7	1.6	12.5	ST
48	0.8	0.4	2	1.2	33.33333333	SM
49	0.3	0.8	2.666666667	1.1	27.27272727	SM
50	0.4	1.1	2.75	1.5	26.66666667	SM
51	0.4	0.9	2.25	1.3	30.76923077	SM
52	0.5	1.0	2	1.5	33.33333333	SM

Contd.....

Table 1. Contd.....

53	0.2	0.8	4	1	20	ST
54	1.2	0.4	3	1.6	25	ST
55	0.2	0.7	3.5	0.9	22.22222222	ST
56	0.4	1.2	3	1.6	25	ST
57	0.4	0.5	1.25	0.9	44.44444444	M
58	0.3	1.2	4	1.5	20	ST
59	1.0	1.0	1	2	50	M
60	1.0	1.0	1	2	50	M
61		0.7	0.7			T
62		1.1	1.1			T
63		0.5	0.5			T
64		0.9	0.9			T
65		1.1	1.1			T
66		0.7	0.7			T
67		1.0	1			T
68		0.9	0.9			T
69		1.2	1.2			T
70		0.6	0.6			T
71		0.4	0.4			T
72		0.5	0.5			T
73		0.3	0.3			T
74		0.5	0.5			T
75		0.9	0.9			T
76	1.2	0.6	2	1.8	33.33333333	SM
77	1.0	0.4	2.5	1.4	28.57142857	SM
78		0.7	0.7			T
79		1.1	1.1			T
80		0.7	1.285714286	1.6	43.75	M
81	0.9	0.4	2	1.2	33.33333333	SM
82		0.8	0.8			T
83		0.8	0.8			T
84		0.4	0.4			T
85		0.6	0.6			T
86	0.8	0.4	2	1.2	33.33333333	SM
87		0.4	0.4			T
88		0.9	0.9			T
89		0.7	0.7			T
90		0.9	0.9			T
91		0.8	0.8			T
92	0.3	0.9	3	1.2	25	ST
93	1.1	1.1	1	2.2	50	M
94	0.5	0.7	1.4	1.2	41.66666667	M
95	0.6	0.8	1.333333333	1.4	42.85714286	M
96		0.6	0.6			T
97		0.7	0.7			T
98		1.0	1			T
99		0.9	0.9			T
100	0.4	0.8	2	1.2	33.33333333	SM

Table 2. Karyomorphology of *N. hexastichus* chromosomes.

S.No	p arm (µm)	q arm (µm)	Arm ratio	Total length (µm)	Centeromeric index	Chromosome type
1	0.4	0.4	1	0.8	50	M
2	0.7	0.3	2.333333333	1	30	SM
3	1.1	0.6	1.833333333	1.7	35.29411765	SM
4	0.8	0.7	1.142857143	1.5	46.66666667	M
5	0.5	0.7	1.142857143	1.2	41.66666667	M
6	1.0	0.6	1.666666667	1.6	37.5	M
7	0.5	0.6	1.2	1.1	54.54545455	M
8	0.6	0.3	2	0.9	33.33333333	SM
9	0.5	0.4	1.25	0.9	44.44444444	M
10	0.8	0.6	1.333333333	1.4	42.85714286	M
11	0.7	0.6	1.166666667	1.3	46.15384615	M
12	0.5	0.4	1.25	0.9	44.44444444	M
13	0.8	0.6	1.333333333	1.4	42.85714286	M
14	0.9	0.3	3	1.2	25	ST
15	0.6	0.6	1	1.2	50	M
16	1.0	0.4	2.5	1.4	28.57142857	SM
17	0.4	0.9	2.25	1.3	30.76923077	SM
18	0.6	0.7	1.166666667	1.3	46.15384615	M
19	0.9	0.4	2.25	1.3	30.76923077	SM
20	0.6	0.4	1.5	1	40	M
21	0.2	1.3	6.5	1.5	13.33333333	ST
22	0.3	1.2	4	1.5	20	ST
23	0.4	0.9	2.25	1.3	30.76923077	SM
24	0.5	0.5	1	1	50	M
25	0.4	0.7	1.75	1.1	36.36363636	SM
26	0.7	0.1	7	0.8	12.5	ST
27	0.4	1.2	3	1.6	25	SM
28		1.0	1			T
29		0.4	0.4			T
30		0.6	0.6			T
31		1.3	1.3			T
32		0.7	0.7			T
33		0.9	0.9			T
34		1.0	1			T
35		1.1	1.1			T
36		0.9	0.9			T
37		0.8	0.8			T
38	0.7	0.6	1.166666667	1.3	46.15384615	M
39	0.6	0.8	2	1.2	50	SM
40	0.5	0.9	1.5	1.5	33.33333333	M
41		0.8	0.8			T
42		1.0	1			T
43		0.7	0.7			T
44		0.9	0.9			T
45		1.3	1.3			T
46		1.4	1.4			T
47		0.9	0.9			T
48		1.1	1.1			T
49		1.8	1.8			T
50		1.0	1			T
51		0.6	0.6			T
52		0.8	0.8			T

Contd.....

Table 2. Contd.....

53		1.1	1.1			T
54		0.9	0.9			T
55		1.3	1.3			T
56		1.0	1			T
57		1.1	1.1			T
58		1.5	1.5			T
59		1.1	1.1			T
60		0.7	0.7			T
61	0.4	0.7	1.75	1.1	36.36363636	SM
62	0.4	0.8	2	1.2	33.33333333	SM
63	0.6	0.7	1.166666667	1.3	46.15384615	M
64	0.6	0.4	1.5	1	40	M
65	0.6	0.4	1.5	1	40	M
66	0.6	0.4	1.5	1	60	M
67	0.9	0.6	1.5	1.5	60	M
68	1.2	0.6	2	1.8	33.33333333	SM
69	0.8	0.6	1.333333333	1.4	42.85714286	M
70	0.6	0.5	1.2	1.1	45.45454545	M
71	0.9	0.7	1.285714286	1.6	43.75	M
72	0.5	1.1	2.2	1.6	31.25	SM
73	0.5	0.6	1.2	1.1	45.45454545	M
74	1.1	0.6	1.833333333	1.7	35.29411765	SM
75	0.5	0.6	1.2	1.1	45.45454545	M
76	0.8	0.3	2.666666667	1.1	27.27272727	SM
77	0.8	0.3	2.666666667	1.1	27.27272727	SM
78	0.3	0.6	2	0.9	33.33333333	SM
79	0.5	0.3	1.666666667	0.8	37.5	M
80	0.4	0.6	1.5	1	60	M
81		1.2	1.2			T
82		0.9	0.9			T
83		0.6	0.6			T
84		1.4	1.4			T
85	0.7	0.5	1.4	1.2	41.66666667	M
86	0.4	0.9	2.25	1.3	69.23076923	SM
87	0.7	0.5	1.4	1.2	41.66666667	M
88		1.2	1.2			T
89		0.7	0.7			T
90		0.4	0.4			T
91		0.9	0.9			T
92	1.3	0.6	2.166666667	1.9	31.57894737	SM
93		1.3	1.3			T
94		1.0	1			T
95	0.4	0.5	1.25	0.9	44.44444444	SM
96	0.9	0.8	2	1.7	47.05882353	SM
97	0.9	0.8	1.142857143	1.5	47.05882	M
98		0.7	0.7			T
99		0.7	0.7			T
100	0.7	0.6	1.166666667	1.3	51.42857143	M

pericentric inversions and/or heterochromatic processes (Mani *et al.*, 2013). and the evolution and systematics are considered robust tools in resolving taxonomic uncertainties (Mani *et al.*, 2010).

Conclusion

They are distinctly two different species *N. hexagonolepis* and *N. hexastichus* based on the number of morphologically different chromosomes, which has a support of meristic characteristics. The study will provide insight into using a cytogenetic study to identify the fish species population of mahseer rich flowing rivers of Meghalaya, India. In addition, further analysis of studying chromosome evolution through sophisticated tools such as FISH or Fluorescence in Situ Hybridization karyotype and distribution of constitutive heterochromatin, the findings which can be used in the phylogenetic study of the different species of *Neolissochilus*.

Conflict of interest

The authors declare that they have no conflict of interest.

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