***Actus salvadoricus* Baqri and Jairajpuri (Mononchida: Mylonchulidae) from Japan with comment on the phylogenetic position of the genus *Actus* based on 18S rDNA sequences**

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The nematode genus *Actus* belonging to the order Mononchida, an important group of predatory nematodes, is rare in the world. *Actus salvadoricus* Baqri and Jairajpuri, 1974, which had been described from El Salvador, were found from subtropical forest in the northern part of Okinawa Island, Japan. Its morphological observation is made herein and its phylogenetic position is elucidated based on 18S rDNA sequence. *A. salvadoricus* collected from Okinawa is characterized by having its buccal cavity with a moderately developed dorsal tooth, vertical subventral plates with a single row of denticles arranged as 3:3 or 3:4 or 2:4 on two plates, distinct excretory pore, elongate-conoid ventrally arcuate tail, with tandem caudal glands and terminal spinneret. Based on the nearly complete 18S rDNA sequence data already known and revealed in this work, phylogenetic trees of Mononchida were constructed using parsimony and maximum likelihood algorithms. The results show that the genus *Actus* forms a sister group with the species of the genus *Mylonchulus* in the suborder Mononchida, showing the valid status of its taxonomic group. Jpn. J. Nematol. 38 (2), 57-69 (2008).

Key words : taxonomy, new record, Okinawa Island, phylogenetic tree, primers for PCR.

INTRODUCTION

Nematodes of the order Mononchida Jairajpuri, 1969 are all predaceous in nature and play an important role in regulating nematode population in the soil ecosystem (Jairajpuri and Khan, 1982). Not much is known about the mononchid fauna of Japan. Imamura (1931), Kaburaki (1933), Yokoo and Koga (1966),

Shishida (1998), Nakazawa (1999) and Khan et al., (2000, 2002) described several species of mononchs but still the information is scanty. An intensive survey was conducted in various parts of this country to investigate the mononchid fauna. Among the several genera recorded, we recognized a population belonging to the rare genus *Actus* Baqri and Jairajpuri, 1974 from Yona field subtropical forests in Okinawa Island. With precise observations it was identified as *A. salvadoricus* Baqri and Jairajpuri, 1974.

Genus *Actus* consists of only four known species, *A. minutus* (Mulvey, 1963), the type species, from Saipan and Truk, North Pacific, *A. salvadoricus* Baqri and Jairajpuri, 1974 from El Salvador, Central America, *A. nanurus* Siddiqi

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(1984) from Fiji, South Pacific and *A. neocaledonensis* Yeates, 1992 from New Caledonia. Any other records of this genus were not known except the original description of each species. The original description of *A. salvadoricus* was inadequate from the recent points of view as well as the others were. Here we decided to ascribe several morphological characters of *A. salvadoricus*.

Recently, DNA-based techniques have provided an attractive tool to help clearing up the controversial opinions in nematode taxonomy. The nematode DNA cistron typically consists of several hundred tandemly repeated copies of the transcribed units (small subunit or SSU or 18S; large subunit or LSU or 28S; 5.8S; internal transcribed spacer or ITS and external transcribed spacer) and an external non-transcribed or intergenic spacer (NTS) (Hill and Dixon, 1991). The highly conserved sequences (18S and 28S), and highly variable spacer sequences (NTS and ITS) are considered most useful for phylogenetic inference between very distantly related species for the former, and very closely related species for the latter (Page and Holmes, 1998). In the present study, we have also attempted to assess the molecular phylogenetic position of this rare nematode genus, *viz a viz* other closely related genera in the suborder Mononchina Kirjanova and Krall, 1969 using 18S rDNA sequence data.

MATERIALS AND METHODS

Nematode extraction:

The nematodes were isolated from the soil samples collected from evergreen forest by Cobb's sieving and decantation (Cobb, 1918) and modified Bearmann's funnel technique (Southey, 1970). Specimens obtained in water were killed by pouring equal quantity of boiling TAF, dehydrated by slow method and mounted in anhydrous glycerin (Seinhorst, 1962). Measurements were taken with an ocular micrometer and draw-

ings were made using drawing tube mounted on a Nikon Optiphot 2 research microscope. LM photographs were taken using Olympus digital camera CS230B operated through Dell Precision 370 personal computer.

DNA extraction:

A single nematode was transferred to 1 μ l dH₂O in a 0.2 ml flat top-PCR tube cap, crushed with a sterile needle and then 10 μ l of worm lysis buffer (Orui (1996): 10 mM Tris-HCl: pH 8.0, 1 mM EDTA, 1% IGEPAL CA-630 (Sigma, USA) and 100 μ g/ml Proteinase K) was added. The cap and a tube were connected, and the mixture was shifted into the tube with centrifuge. Tubes were frozen at -45°C for at least one hour, incubated at 65°C (2 hr), and 95°C (10 min) consecutively. After incubation, tubes frozen at -45°C for at least one hour and used for PCR. DNA samples were stored at -45°C until further use.

PCR:

The PCR reaction mixture contained 2.5 U per 100 μ l Prime Star Polymerase HS (Takara, Orsu, JAPAN), 1 $\times$ PCR buffer manufacture, 0.2 mM dNTPs and 0.5 μ M each primer. Genomic DNA, 0.5 μ l, was used directly as template for PCR in 25 μ l of the reaction mixture. A negative control without template DNA was included in each amplification. The forward primer EukF (10) and the reverse primer EukR (10) (Delong, 1992) were used in the PCR (Table 1) which covers nearly the entire area of 18S rDNA. The PCR 18S amplification profile performed on PT-800 thermal cycler (ASTEC, Japan) consisted of 3 min at 98°C; 32 cycles of 10 sec at 98°C, 15 sec at 59°C and 2.5 min at 72°C, followed by a final step of 10 min at 72°C. Primers 664 and 665 (Table 1) were used for ITS amplification (Vrain et al., 1992) which covers ITS1, ITS2, 5.8S and small portions of 18S and 28S. The PCR ITS amplification program consisted 3 min at 98 °C, 28 cycles of 10 sec at 98 °C, 15 sec at 54 °C and 1 min at 72 °C, followed by a

Table 1. Oligonucleotide used in this study for PCR and to elucidate 18S / ITS rDNA sequences.

Oligo name	Primer sequence (5'-3')	Sense
¹ EukF (10)	AACCTGGTTGATCCTGCCAGT	Forward
² SSU18A	AAAGATTAAGCCATGCATG	Forward
³ 9FX	AAGTCTGGTGCCAGCAGCCGC	Forward
³ 24F1	AGAGGTGAAATTCTTGGATC	Forward
³ 2FX	GGAAGGGCACCACCAGGAGTGG	Forward
² SSU23F	ATTCCGATAACGAGCGAGA	Forward
⁴ 664	TTGATTACGTCCTGCCCTTT	Forward
⁵ C18	GTTTCCGTAGGTGAACCTGC	Forward
² SSU9R	AGCTGGAATTACCGCGGCTG	Reverse
² SSU26R	CATTCTTGGCAAATGCTTTTCG	Reverse
² SSU23R	TCTCGCTCGTTATCGGAAT	Reverse
¹ EukR (10)	TGATCCTTCTGCAGGTTTCACCTAC	Reverse
⁴ 665	TTTCACTCGCCGTTACTAAGG	Reverse
⁵ C26	ATATGCTTAAGTTCAGCGGGT	Reverse

Sources: ¹ Delong (1992) ² Blaxter et al. (1998) ³ Meldal et al. (2007)⁴ Vrain et al. (1992) ⁵ Curran et al. (1994)

final step of 10 min at 72 °C. Amplified PCR product, 5 µl, was electrophoretically fractionated in 1 x TAE buffer in 2% agarose gel and visualized by staining with ethidium bromide. Gels were viewed on a UV transilluminator. The remaining PCR product stored at -45°C for further use.

DNA Sequencing:

PCR products, 2 µl, were purified with the 40 times diluted ExoSAP-IT® (USB Co., USA) 3 µl, by incubating the mixture for 90 min at 37°C, followed by enzyme inactivation for 10 min at 80°C. Purified PCR products were used for DNA sequencing using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) with a reaction kit (Big Dye Terminator Cycle

Sequencing Ready Reaction Kit ver. 3.1, Applied Biosystems, Japan). In addition to the amplification primers, other sequencing primers (Table 1) were also used to ensure proper overlapping of fragment products from both strands to obtain sequences of nearly entire 18S and complete ITS rDNA regions (Fig. 1).
Phylogenetic Analysis:

DNA sequence data from various primers were assembled using GENE-TYX-WIN version 1.03 (Software Development, Japan). Multiple alignments of the known and new sequences of 18S rDNA were performed in ClustalX version 1.8 (Thompson et al., 1997) with default parameter settings and further edited manually. All phylo-

genetic trees were constructed in PAUP* version 4.0b10 (Swofford, 2002) using maximum parsimony (MP) and maximum likelihood (ML) criteria. MP analysis used the heuristic search option with tree bisection-reconnection (TBR) branch swapping with 10 random sequence additions of taxa. Gaps were scored as "missing". ML analysis was also performed using the heuristic search option with TBR branch swapping and 10 random sequence additions of taxa. The most appropriate model of sequence evolution for our ML analysis was selected using the Akaike Information Criterion (AIC) as implemented in Modeltest version 3.7 (Posada and Crandall, 1998). Support for each internal branch of the optimal trees was assessed *via*

Fig. 1. Schematic diagram showing relative positions of the primers for PCR and sequencing primers to provide data from 18S and ITS rDNA segments. Sizes are approximate not to the scale.

1,000 nonparametric bootstrap replicates in PAUP*. Phylogenetic trees were examined using TreeView (Page, 1996) and converted into graphic file for Adobe Illustrator 9 (Adobe Systems Inc., USA).

RESULTS

Actus salvadoricus Baqri and Jairajpuri, 1974

Measurements: See Table 2.

Morphology (Fig. 2 and 3):

Females (n = 3): Body slightly curved ventrad upon fixation. Cuticle smooth, 2-3 µm thick along the body. Lateral chords about one-third of body width at mid body. Lateral, dorsal and ventral body pores indistinct. Lip region offset, distinctly wider than adjoining body, about

three times as wide as high and about half as wide as body width at neck base. Labial papillae projecting slightly above labial contour. Amphids cup-shaped with slit-like aperture, slightly anterior to the dorsal tooth apex. Buccal cavity thick-walled, barrel-shaped, slightly narrow at base, about 1.9-2.0 times as long as wide. Dorsal tooth moderately developed, anteriorly directed, its tip at 83-87% from base of stoma. Vertical subventral plates with denticles arranged as 3:3, 3:4, 2:4 on two plates in three females examined. The denticles near the base of vertical plates distinctly larger than the upper ones, rather a gradual decrease in size from lower to upper ones. Oblique subventral walls of buccal cavity with two foramina each. Nerve

Table 2. Measurements of *Actus salvadoricus* (all measurements in µm).

Characters	El Salvador population	Japan Population	
	(After Baqri & Jairajpuri, 1974) n=4	Females n = 3	Juvenils n = 3
Body length	850-1210	1168-1268 (1215)	1074-1118 (1102)
Body width	-	45.0-47.5 (46.1)	37.1-37.9 (37.9)
a	20-24	25.6-26.8 (26.3)	28.9-29.3 (29.0)
b	3.5-4.3	3.7-3.9 (3.8)	3.7-3.9 (3.8)
c	17-20	21.0-24.3 (22.8)	21.5-23.7 (22.4)
c'	1.8-2.4	1.9-2.2 (2.1)	1.9-2.2 (2.1)
V (%)	61-64	62.8-63.9 (63.4)	-
G1	-	13.2-15.7 (14.5)	-
G2	-	9.4-14.7 (11.3)	-
Lip region width	23-24	23.5-24.5 (24.2)	20.5-21.3 (20.8)
Lip region height	8-9	6.0-6.3 (6.2)	5.1-5.5 (5.4)
Buccal cavity length	24-26	27.6-27.7 (27.7)	23.3-23.7 (23.6)
Buccal cavity width	14-15	13.5-15.1 (14.3)	11.1-12.2 (11.6)
Position of dorsal tooth from base of stoma	18	20.4-20.9 (20.6)	17.4-18.2 (17.8)
Nerve ring from anterior end	78-99	104-115 (110)	89-96 (92)
Neck length	-	300-326 (316)	290-294 (292)
Anterior genital branch	-	118-200 (158)	-
Posterior genital branch	-	118-183 (150)	-
Vulva position from anterior end	-	734-810 (771)	-
Rectum length	20-23	22.1-23.7 (22.6)	20.5-21.3 (21.0)
Anal body diameter	-	24.8-27.6 (25.8)	22.5-24.9 (23.7)
Tail length	51-61	52.2-55.7 (53.4)	47.1-52.1 (49.4)

Fig. 2. *Actus salvadoricus*. A: Entire female, B–D: Head ends showing teeth arrangements and amphid position, E: Anterior region, F: Vulval region, G: Female genital system, H: Posterior end.

Fig. 3. *Actus salvadoricus*. A: Head ends showing dorsal tooth, B-C: Head ends showing denticle arrangements (through focus series), D: Head end showing amphid position, E: Excretory cell (arrow), F: Oesophago-intestinal junction, G: Vulval region, H: Tail showing spinneret. Scale bar is 10 µm.

ring at 32-35% of neck length from anterior end. Excretory pore distinct, at 35-37% of neck length from anterior end. An excretory cell observable, directly connected to the excretory pore. Orifice of pharyngeal glands located as follows: dorsal 136-141 µm from anterior end; first pair of subventrals 64-71 µm from the orifice of dorsal one; second pair 65-74 µm from the first pair. Pharyngo-intestinal junction non-tuberculate. Genital system amphidelphic; both sexual branches almost equally developed. Ovary reflexed, measuring 69-138 µm (anterior) and 115-157 µm (posterior) with oocytes arranged in a single row except near tip. Oviduct with distinct *pars dilatata*. Uterus filled with single oocyte, measuring 108-121 × 36-29 µm in two specimens. Sphincter indistinct. No trace of sperms either in uterus or oviduct. Vagina extending about one-fourth of the corresponding body width; *pars proximalis vaginae* 6.5-7.0 µm long, encircled by circular muscles; *pars refringens vaginae* with triangular sclerotization, each measuring 3.5-4.0 × 2.5 µm, cw 5.0-6.0 µm; *pars distalis vaginae* 2.0 µm. Rectum 0.8-

0.9 times the anal body width long. Tail elongate-conoid, ventrally arcuate, 1.9-2.2 anal body widths long. Caudal glands tandem; spinneret terminal.

Juveniles (n = 3): Similar to female in general morphology. Vertical subventral plates of buccal cavity with denticles arranged as 2:3, 3:3, 3:4. Caudal glands and spinneret well developed.

Male: Not found.

Specimens examined: Three females and 3 juveniles collected from soil around trees from evergreen forest, Yona Field, Subtropical Field Sciences Center, Faculty of Agriculture, University of the Ryukyus, Okinawa Pref. Okinawa Island, Japan (29. xi. 2006, M. Ochia leg.). Specimens are deposited at the nematode collection of the Department of Zoology, Aligarh Muslim University, India.

Diagnosis and relationship: Female length 1.2-1.3 mm; stoma 13-15 × 27-28 µm; dorsal tooth apex at 83-87% of stoma length; vulva at 62-64%; gonads paired; no vulval papillae; tail elongate conoid, ventrally arcuate, 1.9-2.2 anal

Fig. 4. Maximum likelihood tree obtained from heuristic search of 18S rDNA sequence data for the known and newly sequenced operational taxonomic units in the suborder Mononchina. Bootstrap support values for clades occurring at >50% frequency are shown. A numerical sign indicates sequence data is from this study. See text for details of ML analysis.

body widths long; caudal glands well developed with distinct terminal opening. Male unknown.

Our three specimens have anteriorly positioned amphids, anteriorly directed and moderately developed dorsal tooth, longer tail and pronounced spinneret, which fully agree with the description of *A. salvadricus* provided by Baqri and Jairajpuri 1974. It can be pointed that their denticles show a gradual decrease in size from lower to upper. *Actus salvadricus* Baqri and Jairajpuri, 1974 closely resembles *A. minutus* in the amphid position (at the level of dorsal tooth apex) and body dimensions, but is distinguished in the anterior position of amphids (more anterior), the shape of dorsal tooth (distinctly anteriorly directed with anterior wall concave), and in having a comparatively longer tail with a pronounced terminal opening of caudal glands. From *A. nanurus* Siddiqi, 1984, it differs in having comparatively longer and slender body (vs. 0.9-1.1 mm; $a = 22-28$), and longer tail (vs. $c = 33-57$; $c' = 0.8-1.3$). From *A. neocaledonensis* Yeates, 1992, the species differs in having comparatively smaller body size (vs. $L=1.21-1.47$ mm); smaller stoma size (vs. 31-36 μm); dorsal tooth more anteriorly placed (vs. 55-70% of stoma length); comparatively posterior vulva (vs. $V = 60-63$), and conspicuous caudal gland opening (vs. caudal gland opening obscure).

Molecular profiles and phylogeny:

All unique sequences acquired in this study were deposited in GenBank by Accession Nos: AB361035 (SSU) and AB361450 (ITS) for *Acutus salvadoricus*; AB361451 (SSU) for *Mononchus truncatus*; AB361452 (SSU) for *Clarkus palliatus* (Fig. 4, # codes).

Sequences were compared with existing sequences in GenBank database via BLAST pairwise searches to determine the nearest match on the likelihood tree (Fig. 4). The nearly complete SSU sequences obtained from all the included taxa consisted of 1,766 (*Anatonchus tri-*

dentatus) to 1591 (*Miconchus* cf. *faciatus*) bp and the edited ClustalX alignments contained 1498 bp.

The best-fit substitution model for the ML analysis under AIC was GTR + + I, that is, the general time-reversible model with a gamma correction for variable rates among sites (shape parameter = 0.7267) and with incorporating a proportion of invariant sites (proportion = 0.559). The ML analysis found the optimal tree ($-\ln L = 4487.39836$) (Fig. 4). The bootstrap support for the ML tree is shown in Fig. 4.

The MP analysis using 18S rDNA sequences also conducted (Data not shown). The MP analysis revealed 12 equally most parsimonious trees with a length of 420 steps (98 characters are parsimony-informative, CI (consistency index) = 0.748, RI (retention index) = 0.836). All optimal trees are nearly identical in topology except for several terminal differences which resulted in trichotomies in the strict consensus tree. The bootstrap support for each branch of the consensus tree shows that almost all clades are statistically reliable. The consensus tree shows that there was no difference with the ML tree (especially for the clade which includes the genus *Actus*) except for some outer clades with lower bootstrap values.

The ML tree (Fig. 4) shows four major clades in our studied taxa. Members of the genus *Mononchus* (Clade I) make an independent clade with high bootstrap support while *Mylonchulus* species, along with *Actus salvadoricus* as a sister group, form another clade (Clade II). The third clade (Clade III) includes members of the subfamily Prionchulinae like *Coomansus parvus*, *Clarkus papillatus*, *Prionchulus muscivorus* and *Prionchulus punctatus*, and the last clade (Clade IV) is composed of the tuberculate mononchs comprising *Anatonchus tridentatus* and *Miconchus* sp. The present 18S rDNA sequence data set clearly shows that the genus

Actus stands neither with Prionchulinae nor with *Mononchus* spp. and is considered closely related to *Mylonchulus* spp. within Clade II. Our hypothesis of the phylogenetic position of the genus *Actus* is corroborated by relatively high bootstrap support values for these major clades.

DISCUSSION

Actus salvadoricus, a valid species

Baqri and Jairajpuri (1974) proposed the genus *Actus* for *Sporonchulus minutus* (Mulvey, 1963) and a new species, *A. salvadoricus* which they described from El Salvador in Central America. It was stated that members of *Actus* have on each subventral wall a single row of few (4-5) denticles which is in contrast to other species of *Sporonchulus* (Cobb, 1913) Pennak, 1953 where additional rows of irregularly scattered denticles are present. They (*l.c.*) differentiated it from *Prionchulus* in the absence of longitudinal rib along which the subventral denticles are arranged, and placed it under Mononchidae Chitwood, 1937 close to the genus *Prionchulus* (Cobb, 1916) Wu and Hoeppli, 1929. Subsequently, Jairajpuri and Khan (1982) placed it under Prionchulinae Andrssy, 1976. Siddiqi (1984) described a third species, *A. nanurus* from Fiji and argued against placing *Actus* in Prionchulinae, considered it to be more close to *Sporonchulus* and a member of the subfamily Mononchinae Chitwood, 1937. Yeates (1992) added a further new species *A. neocaledonensis* from New Caledonia.

Baqri and Jairajpuri (1974) described *A. salvadoricus* from El Salvador and differentiated it from *A. minutus* (Mulvey, 1963) Baqri and Jairajpuri (1974) in the anterior position of amphids, the shape of dorsal tooth, and in having a comparatively longer tail with a pronounced terminal opening of caudal glands. Mulvey (1963) described *A. minutus* (= *Sporonchulus minutus*) from Saipan based on

three females and three juveniles. As per his description and figure the dorsal tooth is medium sized and opposed by longitudinal rows of denticles. The posterior two denticles are nearly basal and larger than other eight, *i.e.* 4+1 (5) denticles are on each plate and an obscure terminal opening on tail. Andrssy (1993) synonymized *A. salvadoricus* with *A. minutus* giving the reason that *A. salvadoricus* differs from *A. minutus* only in location of amphids which lie a little more anterior. He did not mention the difference in the shape of dorsal tooth or the pronounced terminal opening of the caudal glands. The dorsal tooth in our specimens as well as in original description of *A. salvadoricus* (fig. 1B of Baqri and Jairajpuri (1974)), is distinctly anteriorly directed with its anterior wall becoming concave (*vs.* the dorsal tooth not anteriorly directed, its front edge straight, perpendicular to the body axis in *A. minutus* (Fig. 10 of Mulvey (1963))). Another important difference between the two species is in the number of denticles on two subventral walls which is five on each plate in *A. minutus*, whereas, it is five on one plate and four on the other in type specimens (Fig. 1C of Baqri and Jairajpuri (1974)). In our three specimens, the denticles on two plates were 3:3, 3:4, 2:4, *i.e.* they have tendency of having different number of denticles on the subventral walls. In *A. minutus* the posterior denticle is larger than the other four. In *A. salvadoricus* also the posterior denticle is distinctly larger than the others where we see a gradual decrease in size from lower to upper. This is not the case with *A. minutus* where all the denticles are of same size or rather the upper two are larger than the lower two if we see the Fig.10 of Mulvey (1963). The most striking difference between the two species is the terminal caudal opening which is well developed in *A. salvadoricus* (Fig. 1F of Baqri and Jairajpuri (1974) as well as in all our specimens from Japan) whereas it is obscure in *A.*

minutus. On the basis of above morphological differences we consider *A. salvadoricus* a valid species.

Phylogenetic analysis:

We recognized four major clades of mononchs in our study, which mostly were in accordance with their morphological classification (Table 3 and Fig. 4). Clade I of our ML tree consisted of the members of genus *Mononchus*. Clade II mainly consisted of the members of genus *Mylonchulus* with *Actus salvadoricus* as a sister group. These two genera belong to family Mylonchulidae. Clade III were made of Genera *Prionchulus*, *Clarkus* and *Coomansus*, which belong to Family Mononchidae, Subfamily Prionchulinae. Members of Family Anatonchidae, Subfamily Anatonchinae, *Anatonchus* and *Miconchus* were in the Clade IV.

The results of our study also indicate two groups under Mononchidae viz., Mononchinae represented by the genus *Mononchus* (clade I) and Prionchulinae (clade III). Members of Prionchulinae have two apomorphies, possession of a ridge on subventral plate and absence of caudal glands and spinneret whereas, Mononchinae lack ridge on subventral plate and have well developed caudal glands and spinneret.

Baqri and Jairajpuri (1974) while discussing the systematic position of the genus *Actus*, wrote "the differences from *Sporonchulus* are considered to be of more fundamental nature and thus the new genus has been removed from this group (i.e., Sporonchulinae, Mylonchulidae Jairajpuri, 1969), and placed under the family Mononchidae Chitwood, 1937 close to the genus *Prionchulus*". As per Jairajpuri (1969) Mylonchulidae is divided into two subfamilies, viz, Mylonchulinae Jairajpuri, 1969 with the genera *Mylonchulus* (Cobb, 1916) Altherr, 1953 and *Polyonchulus* Mulvey and Jensen, 1967, and the subfamily Sporonchulinae Jairajpuri, 1969 with

the genera *Sporonchulus* (Cobb, 1917) Pennak, 1953, *Granonchulus* Andrssy, 1958, *Prionchuloides* Mulvey, 1963, *Judonchulus* Andrssy, 1958, and *Brachonchulus* Andrssy, 1958. Jairajpuri and Khan (1982) placed *Actus* in the subfamily Prionchulinae Andrssy, 1976 under the family Mononchidae. Siddiqi (1984) considered *Actus* close to *Sporonchulus* under the subfamily Mononchinae. It means, he did not accept the two subfamilies Mylonchulinae and Sporonchulinae under Mylonchulidae and considered *Sporonchulus* under Mononchidae rather than Mylonchulidae. Andrssy (1993) accepted only two families under Mononchoidea, viz., Mononchidae with the subfamilies Mononchinae Chitwood, 1937 and Cobbonchinae Jairajpuri, 1969 and Mylonchulidae with the single subfamily Mylonchulinae. He grouped *Actus* under the subfamily Mononchinae close to *Prionchulus*, *Clarkus*, *Sporonchulus* etc. The present analysis of 18SrDNA sequence indicating close relationship of *Actus* with *Mylonchulus* places it in the family Mylonchulidae Jairajpuri, 1969. The nature of its buccal dentation, the subventral denticles arranged in a row in *Actus* while irregularly scattered in *Sporonchulus*, suggests that *Actus* has close resemblance with *Sporonchulus* as also indicated by Siddiqi (1984) rather than *Prionchulus*. Unfortunately sequence data is not available for any species of *Sporonchulus* nor we were able to collect specimens representing this genus for our study but it is most likely that *Sporonchulus* also belongs to this group as originally conceived by Jairajpuri (1969) with two subfamilies, viz., Mylonchulinae and Sporonchulinae under Mylonchulidae.

The genus *Granonchulus* has been assigned morphologically in Mononchoidea, Mylonchulidae, Sporonchulinae (Jairajpuri, 1969). In our morphological study it is shown that the genus *Actus* has close resemblance with

genus *Sporonchulus*. On the other hand, genus *Granonchulus* occupied a quite different position in the phylogenetic tree using DNA sequences. Further study is needed to clarify the relationship between the genera *Actus* and *Granonchulus*.

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