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DIVERSITY PROFILE OF PROTISTS FLAGELLATES ISOLATED FROM HINDGUT OF *Heterotermes indicola* WASMANN (BLATTODEA: RHINOTERMITIDAE) IN PAKISTAN

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Termites cause a serious menace to wood structures all over the world. They rely mostly on the entozoic fauna for the digestion of cellulosic materials. The present study is based upon the diversity of flagellates protists isolated from the gut of a lower termite, *Heterotermes indicola,* belonging to three genera i.e. *Holomastigotes* (*H. campanula, H. annandalei* and *H. metchnikowi*), *Holomastigotoides* (*H. hemigynum, H. hartmanni, H. kempi, H. koidzumi* and *H. metchnikowi)* and *Pseudotrichonympha* (*P. grassii*). The largest and most abundant species *Pseudotrichonympha grassii* was identified by molecular studies using the SSU rRNA gene, confirmed by phylogenetic analysis and compared with that of the *P. grassii* isolates reported from other parts of the world. The results showed that the *P. grassii* observed in our study was phylogenetically most closely related to the Japanese *P. grassii* isolate. The biodiversity of the entozoic flagellates is important in targeting for biological control of termites as well as for isolation and culturing of flagellates to produce cellulases, an important industrial enzyme.

Keywords: Biodiversity, *Heterotermes indicola*, entozoic flagellates, *Holomastigotes, Holomastigotoides, Pseudotrichonympha.*

INTRODUCTION

Heterotermes indicola (Blattodea: Rhinotermitidae) is a lower termite which harbor entozoic flagellates for cellulose digestion thus causing considerable damage to forests and wooden materials throughout the world except Antarctica. About 434 flagellate's species have been identified in the hindgut of different termite species which belong to one of the three orders: Trichomonadida, Hypermastigida and Oxymonadida (Inoue *et al.,* 2000). During the last two decades termite gut flagellates have been classified using molecular methods, which are considered to be good tools for species identification and for defining evolutionary inferences (Yang and Rannala, 2012). Saldarriaga *et al.* (2011) described the morphology and phylogeny of two *Pseudotrichonympha* species i.e. *P. hertwigi* from *Coptotermes testaceus* and *P. paulistana* from *Heterotermes tenuis*. Phylogenetic analysis of fifteen species of *Pseudotrichonympha* was described by Noda *et al*. (2007) from different termite species. However, he suggested that only one species of *Pseudotrichonympha* is found in a termite species at one time. Thus the evolutionary history of *Pseudotrichonympha* is important not only due to its host specificity but as a host of bacterial endosymbionts, which account for more than two third of the total bacterial population in the termite gut (Noda *et al.,* 2007).The endomicrobes found in the hindgut of termites play a major role in cellulose digestion and manufacture cellulases that break down cellulose into acetate, propionate and butyrate. They also fix nitrogen that gets incorporated into the termite's tissues, excrement and secretions (Mathew *et al*., 2012). Recycling of uric acid by the termite gut bacteria is another source of nitrogen (Thong-On *et al.,* 2012). With the aid of these endomicrobes, such small insects (termites) cause damage worth billions to woods and wooden materials. The information pertaining to protozoa, found in the termites as symbionts, will be helpful towards designing strategies for the production of cellulases and control of lower termites via the development of drugs targeting these protozoa.

Owing to their importance in the termite hindgut, information about these endomicrobes and their diversity is extremely important. The present work was aimed to investigate the diversity of flagellated protozoa with a special focus on *P. grassii*, the largest and the most abundant protozoan found in the hindgut of *H. indicola*. Moreover, to control the population rate of termites and to protect the huge wood damage and economic loss, it is necessary to eliminate the population of gut flagellates which play an important role in cellulose digestion of termites and made symbiotic relationship with each other.

MATERIALS AND METHODS

***Study area and Collection of termites*:** Workers of *H. indicola* were collected from the metropolitan areas of Islamabad (33.729388°N, 73.093146°W) (Fig. 1) Pakistan from March to May 2017. Termites were kept in Petri dishes at 30°C and fed on moistened blotting paper for a week prior to the experiment and identified by using key (Akhtar, 1983). This helped in clearing debris and wood particles from flagellates for distinct morphology.

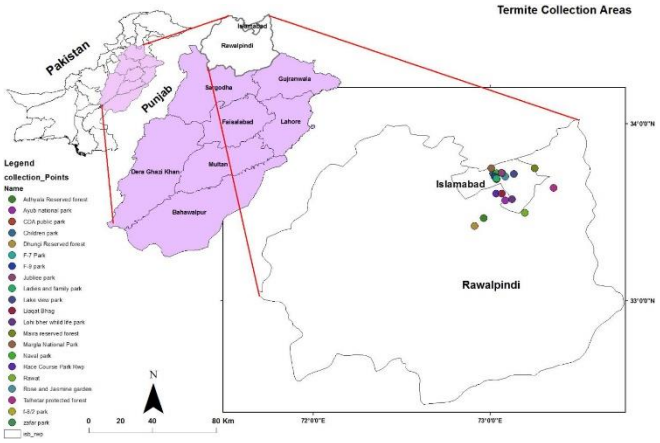


Figure 1. Termites collection sites from Islamabad, Pakistan.

***Light microscopy*:** The hindgut contents of termites were opened in a drop of 0.2% normal saline and tinged with gram iodine solution. Flagellates were observed by using Optika trinocular digital microscope (Optika B-350, Italy). Images and videos were captured in real time with a 12 megapixels digital CCD camera on a trinocular Optika microscope.

***Single cell isolation of P. grassii*:** The gut of termite workers was opened in a 5µl of filter-sterilized buffer (0.1 M NaC1, 10mM Na_3PO_4, pH 6.9) in the cavity slide. From this mixture of gut contents and the buffer, 1ml was taken on another cavity slide and diluted further by adding 4ml of the buffer. This dilution was repeated four times, 1ml of the final diluted contents was used for observations. The flagellates were examined under a digital biological microscope. Individual *P. grassii* cells, identified on the basis of morphology, were picked with a micropipette and collected in PCR tubes.

***PCR amplification and sequencing*:** The cells were directly used as a template in a PCR to amplify their nearly full length SSU rRNA gene. The PCR was performed on five cells. An initial PCR was done by using set of forward and reverse primers (Table 1). The reaction mixture consisted of template cells, dNTP mix (0.2 mM), primers (1µM each), PCR Buffer (1X), *Ex-Taq* DNA polymerase (2.5U) and $MgCl_2$ (1.5mM). Amplification was consisted of 35 cycles of 1 min at 92°C, 1 min at 50°C and1.5 min at 72°C. Agarose gel electrophoresis of the reaction revealed no bands. Therefore, using the first reaction contents as a template, a nested PCR was performed. 25µl of PCR products was added with the same constituents as used for primary PCR by using the second set of primers for nested PCR (Table 1).

The pieces of gel containing the DNA band were excised from the gel and subjected to DNA purification using the Gene JET Gel Extraction Kit (Cat No. K0691, MBI Fermentas). The pGEM-T-Easy vector (Cat No. A1360, Promega) was used to clone the purified products and transformed into DH5α *E. coli* cells. This was followed by plasmid DNA extraction and DNA sequencing of the cloned PCR product.

***Phylogenetic analysis*:** For the phylogenetic investigation, we analyzed our indigenous *P. grassii* taxon and compared it with that of isolated from the other localities of the world. The details are given in Table 4. The sequence alignment and data matrix construction were done in the software Geneious® version 6.1 (Biomatters Ltd., New Zealand). Neighbor joining analysis was performed using the DNA evolution model (Tamura and Nei, 1993) in the Geneious build algorithm. Wagner parsimony (Farris, 1970) and Maximum likelihood (Guindon and Gascuel, 2003) analysis were carried out using PAUP (Swofford, 2002) Geneious plugin. Bayesian analysis was done using MrBayes (Huelsenbeck and Ronquist, 2001) Geneious plugin. Finally, based on the above analysis a consensus evolutionary tree was generated.

RESULTS

***Flagellates biodiversity*:** The protozoan fauna isolated from hindgut of *H. indicola* were categorized according to Adl *et al.* (2005). We identified nine species of flagellates belonged to three genera *Holomastigotes*, *Holomastigotoides* and *Pseudotrichonympha*.

***Taxonomic classification of flagellates isolated from H. indicola*:**

***Class Mastigophora (Diesing)*:** Member of class Mastigophora usually have one to several flagella, the nucleus is vesicular with endosomes.

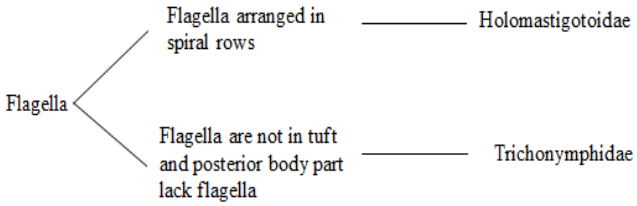
Table 1. Set of primers used for primary and nested PCR.

Primer Name		Sequence	Reference
Primary PCR	Eukl8	5'-TGAGGATCCMGGTTGATYCTGCC-3'	Ohkuma *et al. (*2000)
	Euk1627	5'-CCGAAGCTTACGGGCGGTGTGTRC-3'	
Nested PCR	Har-F	5'-GCGCTACCTGGTTGATCCTGCC-3'	Harper *et al.* (2009)
	Har-R	5'-TGATCCTTCTGCAGGTTCACCTAC-3'	

***Sub Class Zoomastigia*:** These may be free living or parasitic and their body lacks chromatophores. Ensysment is usually common.

***Order Hypermastigida (Grassii and Foa)*:** Flagellates belonging to the order Hypermastigida have numerous flagella and complex cytoplasmic organization.

KEY TO FAMILY



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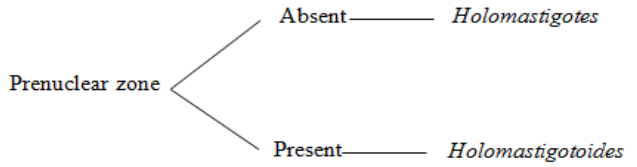
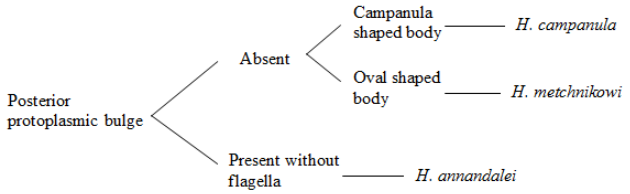


Genus *Holomastigotes* was described by Grassii in 1882 for the 1st time, later on by de Mello in 1927, Kudo in 1947 and Saleem in 1952.

Diagnosis: Nucleus not surrounded by dense protoplasm; basal granules are present deep in the cytoplasm and form deeply stained spiral bands which cover most of the body; flagella arise from basal granules; bulge may or may not be present at posterior end of body; a long axostyle present; body covered with periplast.

KEY TO SPECIES



***Holomastigotes campanula*:**

***Diagnosis*:** Campanula shaped body; broadly round at the anterior end; truncated posteriorly; body covered with flagella in the form of spiral bands; prenuclear zone absent; axostyle long arises near the nucleus and extend towards the posterior end (Fig. 2, Table 2 & 3).

***Holomastigotes metchnikowi*:**

***Diagnosis*:** Cylindrical body; rounded interiorly; no protoplasmic bulged; Nucleus at the anterior end; chromatin material at the periphery; spirally arranged flagella are present on all over the body; cytoplasm differentiated into ectoplasm and endoplasm (Fig. 2, Table 2 & 3).

***Holomastigotes annandalei*:**

***Diagnosis*:** Body oval shaped with broadly round anterior end; nucleus anterior in position; chromatin in the form of 2 to 3 patches; axostyle present; flagella of uniform size present

Table 2. Synonym, type host and additional host of the representative species reported from literature.

Name of species	Synonym	Type host	Add Host
Holomatigotes campanula	*Leidya campanula* (De Mello, 1927) *Holomastigotiodes campanula* (De Mello, 1927) *Holomastigotes campanula* (Saleem, 1952)	*Heterotermes indicola* (Portergaise)	*Heterotermes indicola* (Islamabad)
Holomatigotes metchnikowi	*Leidya metchnikowi* (Franca, 1919) *Holomastigotoides gigas* (De Mello, 1927)	*Heterotermes indicola* (Portergaise)	*Heterotermes indicola* (Islamabad)
Holomatigotes annandalei		*Heterotermes indicola* (Lahore)	*Heterotermes indicola* (Islamabad)
Holomastigotoides metchnikowi	*Leidya metchnikowi* (Franca, 1919) *Holomastigotoides metchnikowi* (De Mello, 1927)	*Coptotermes heimi*	*Heterotermes indicola* (Islamabad)
Holomastigotoides Kempi	*Leidya kempi* (De Mello, 1919)	-	*Heterotermes indicola* (Islamabad)
Holomastigotoides hemigynum	*Holomastigotoides hemigynum* (Grassii, 1917) *Holomastigotoides hemigynum* (De Mello, 1927) *Holomastigotoides hemigynum* (Saleem, 1952) *Leidya annadalei* (De Mello, 1919)	*Coptotermes lecteus* (Austerlia)	*Lecuotermes indicola* (Brazil) *Heterotermes indicola* (Portergaise) *Coptotermes heimi* (Lahore) *Heterotermes indicola* (Islamabad)
Holomastigotoides hartmanni	*Holomastigotoides hartmanni* (Koidzumi, 1921) *Holomastigotoides H. hartmanni* (De Mello, 1927) *Holomastigotoides hartmanni* (Saleem, 1952)	*Heterotermes indicola* (Portergaise) *Heterotermes indicola* (Lahore)	*Heterotermes indicola* (Islamabad)
Pseudotrichonympha grassii	-	*Coptotermes formosanus* *Coptotermes heimi*	*Heterotermes indicola*

Table 3. Sample T-test for the morphometric diversity of flagellates isolated from *Heterotermes indicola*.

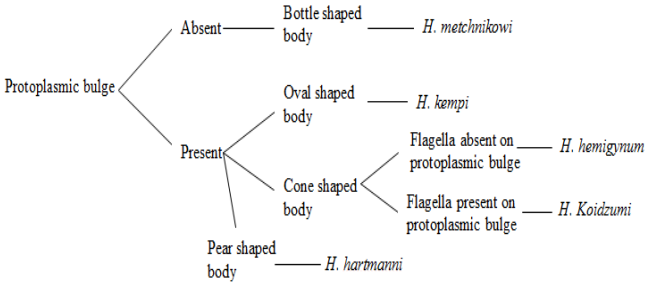
Species	Parameters	N	O.R	$\bar{x}$	S.D	S.E	95% CI
Holomastigotoides kempi	Body Length	25	17.45-132.0	65.46	15.46	8.58	47.36-83.56
	Body width	25	11.9-108.00	51.95	12.65	7.56	36.00-67.89
	Nucleus Diameter	25	8.38-15.00	10.45	2.05	0.48	9.43-11.47
	Flagella Length	25	4.76-96.00	39.86	26.76	6.31	26.56-51.17
	Bulge Length	25	4.76-62.70	32.64	19.07	4.50	23.15-42.12
	Bulge width	25	9.52-96.00	39.61	25.99	6.13	26.68-52.53
Holomastigotoides metchnikowi	Body Length	24	20.04-692.0	122.4	34.80	11.40	36.7-189.80
	Body width	24	19.84-618.0	123.8	40.80	10.60	38.3-199.80
	Nucleus Diameter	24	4.05-83.00	20.25	4.40	2.80	7.67-18.46
	Flagella Length	24	2.00-71.38	15.07	7.50	2.50	6.13-14.45
Holomastigotoides hartmanni	Body Length	12	15.08-74.26	47.90	22.76	8.05	28.87-66.92
	Body width	12	9.28-22.00	16.82	5.23	1.85	12.44-21.19
	Nucleus Diameter	12	3.00-23.80	3.50	1.55	0.55	2.20-4.800
	Flagella Length	12	2.19-8.530	5.31	2.17	0.76	3.49-7.120
Holomastigotoides hemigynum	Body Length	18	14.29-43.00	26.91	9.87	2.33	28.87-66.92
	Body width	18	9.81-24.09	16.86	3.89	0.91	19.02-44.54
	Nucleus Diameter	18	2.96-6.040	3.45	0.94	0.22	2.20-3.910
	Flagella Length	18	7.14-11.90	9.33	1.43	0.33	8.62-10.04
Holomastigotoides Koidzumi	Body Length	20	21.23-72.00	49.65	18.70	4.18	40.90-58.40
	Body width	20	18.88-54.00	28.96	12.36	2.76	23.17-34.74
	Nucleus Diameter	20	2.50-10.08	6.23	1.74	0.39	5.41-7.050
	Flagella Length	20	1.98-8.200	3.70	1.40	0.31	3.04-4.350
	Axostyle length	20	3.00-12.00	6.95	3.45	0.77	5.33-8.570
Holomastigotes annandalei	Body Length	15	14.78-62.21	36.25	19.06	6.70	20.31-52.19
	Body width	15	7.65-58.00	29.25	17.68	6.25	14.47-44.03
	Nucleus Diameter	15	3.10-15.39	3.62	1.40	0.49	2.44-4.800
	Flagella Length	15	1.73-6.350	3.50	1.43	0.50	2.29-4.700
	Bulge Length	15	3.40-7.070	7.38	3.66	1.29	4.31-10.44
	Bulge width	15	7.89-22.07	20.38	12.49	4.42	9.93-30.82
Holomastigotes metchnikowi	Body Length	22	16.38-142.0	81.68	43.45	9.26	62.42-100.9
	Body width	22	13.00-64.26	49.20	15.85	3.38	42.17-56.22
	Nucleus Diameter	22	3.45-14.00	9.06	2.38	0.50	8.00-10.11
	Flagella Length	22	2.36-13.00	7.92	3.23	0.69	6.49-9.350
Holomastigotes campanula	Body Length	22	118.0-239.00	157.92	3.23	0.69	6.49-9.350
	Body width	22	9.09-41.00	20.18	10.32	2.20	15.61-24.76
	Nucleus Diameter	22	2.90-9.450	5.02	1.14	0.24	4.51-5.530
	Flagella Length	22	2.00-5.000	2.34	0.64	0.13	2.05-5.620
Pseudotrichonympha grassii	Body Length	22	9.04-689.0	184.90	39.70	7.90	89.7-250.10
	Body width	22	15.30-610.0	158.70	34.36	7.34	76.1-187.30
	Nucleus Diameter	22	2.38-7.700	5.07	1.35	0.28	4.47-5.670
	Flagella Length	22	3.57-8.400	5.44	1.67	0.35	4.75-6.240
	Length of Centroblepharoplast	22	3.23-8.000	4.13	0.77	0.16	3.79-4.480

in the form of dextral spiral bands on all over the body; protoplasmic groove is present at posterior end; No distinction of ectoplasm or endoplasm (Fig. 2, Table 2 & 3).

***Genus Holomastigotoides*:**

Holomastigotoides was described by Grassii and Foa (1911) for the 1st time, later on by de Mello (1927), Kudo (1947) and Saleem (1952).

***Diagnosis*:** Prenuclear zone present, consists of dense cytoplasm; Basal granules found deep in the cytoplasm, in the form of deeply stained spiral bands, cover most of the body; flagella arise from basal granules extend from anterior to posterior end; bulge may or may not be present at the posterior end of body, may be smooth or covered with flagella; long axostyle present; body covered with periplast.



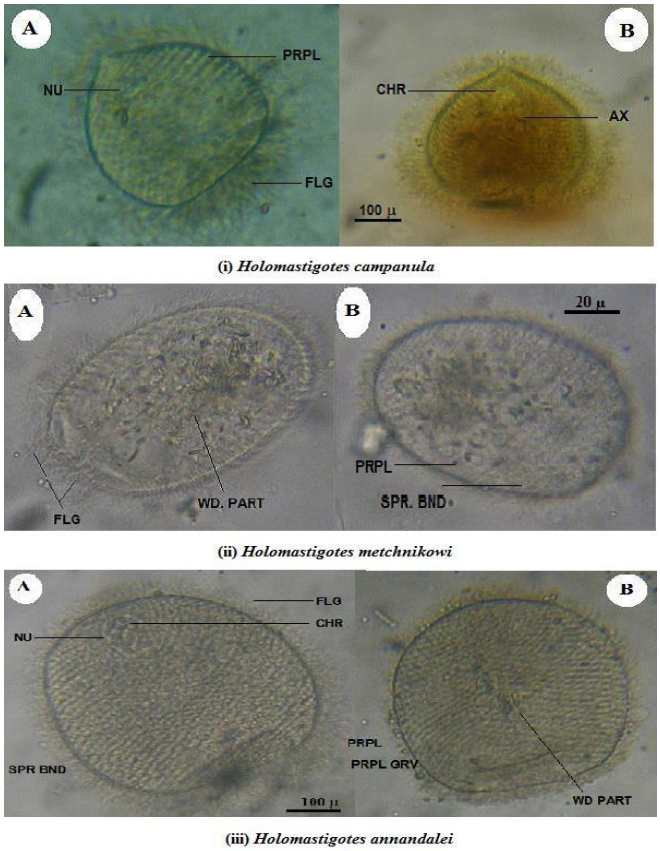


Figure 2. Different microscopic views of (i) *Holomastigotes campanula* (ii) *Holomastigotes metchnikowi* (iii) *Holomastigotes annandalei* at 100X; NU (Nucleus); PRPL (Protoplasm); PRPL GRV (Protoplasmic groove); SPR BND (Spiral bands); CHR (Chromatin); FLG (Flagella); W PART (Wood particles).

KEY TO SPECIES

***Holomastigotoides metchnikowi*:**

***Diagnosis*:** Body bottle shaped; body breadth 1/3rd to 1/4th of the entire length and is uniform till the neck; protoplasmic bulge absent; distinguishable ectoplasm and endoplasm; flagella arise from basal granules in cytoplasm and are arranged in spiral rows; nucleus lies slightly anterior to the middle of the body; chromatin material aggregated to the periphery of the nucleus; axostyle present and projects downward from the nucleus (Fig. 3, Table 2 & 3).

***Holomastigotoides koidzumi*:**

***Diagnosis*:** Body cone-shaped anteriorly; truncated posteriorly; nucleus round and anterior in position; no differentiation of cytoplasm into ectoplasm and endoplasm; prenuclear zone distinct from body wall is arranged into spiral rows; long flagella are present and flagella bands are whorled three times around the body; nucleus surrounds axostyle which extends to posterior (Fig. 3, Table 2 & 3).

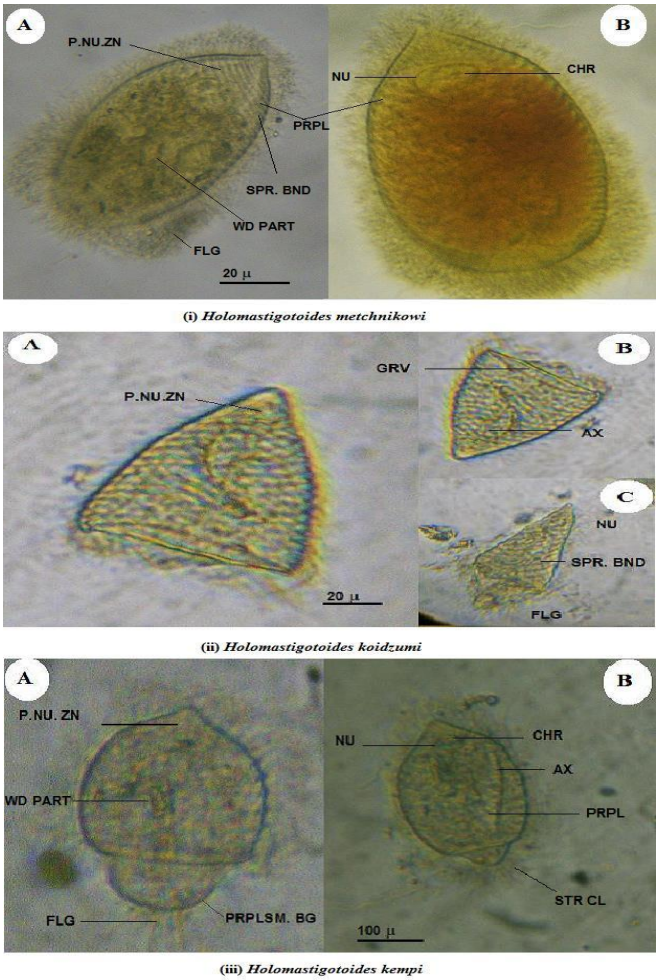
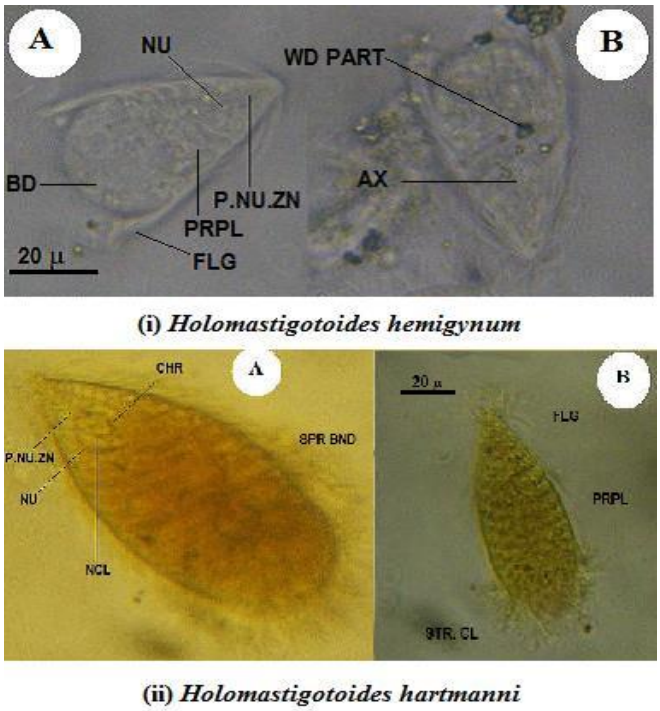


Figure 3. Different microscopic views of (i) *Holomastigotoides metchnikowi* (ii) *Holomastigotoides koidzumi* (iii) *Holomastigotoides kempi* at 100X; NU (Nucleus); PRPL (Protoplasm); PRPL GRV (Protoplasmic groove); SPR BND (Spiral bands); CHR (Chromatin); AX (Axostyle); FLG (Flagella); W PART (Wood particles). P. NU. ZN (Pre-nuclear zone); GRV (Groove); PRPLSM BG (Protoplasmic bulge).

***Holomastigotoides kempi*:**

***Diagnosis*:** Oval shaped body with its anterior part slightly drawn out forming a nipple shape; no differentiation of cytoplasm into ectoplasm and endoplasm; broad protoplasmic bulge present at posterior end of body; flagella cover whole the body; nucleus round in shape; distinct nuclear membrane present; chromatin material arranged in the form of groups or strands; 2 to 3 nucleoli generally visible, their number varies with individuals; axostyle posterior to the nucleus and protrudes downwards; prenuclear zone present (Fig. 3, Table 2 & 3).

***Holomastigotoides hemigynum*:**
***Diagnosis*:** Body cone-shaped; no differentiation of protoplasm into ecto and endoplasm; round nucleus has 1 to 2 nucleoli and covered by a nuclear membrane; chromatin granules arranged in 2 to 3 groups; axostyle projects downwards into the naked protoplasmic bulge from the posterior end of nucleus (Fig. 4, Table 2 & 3).



(i) ***Holomastigotoides hemigynum***

(ii) ***Holomastigotoides hartmanni***

Figure 4. Different microscopic views of (i) *Holomastigotoides hemigynum* and (ii) *Holomastigotoides hartmani* at 100X;
NU (Nucleus); PRPL (Protoplasm); SPR BND (Spiral bands); CHR (Chromatin); AX (Axostyle); FLG (Flagella); W PART (Wood particles) STR CL (Steriocilia); P. NU. ZN (Pre-nuclear zone).

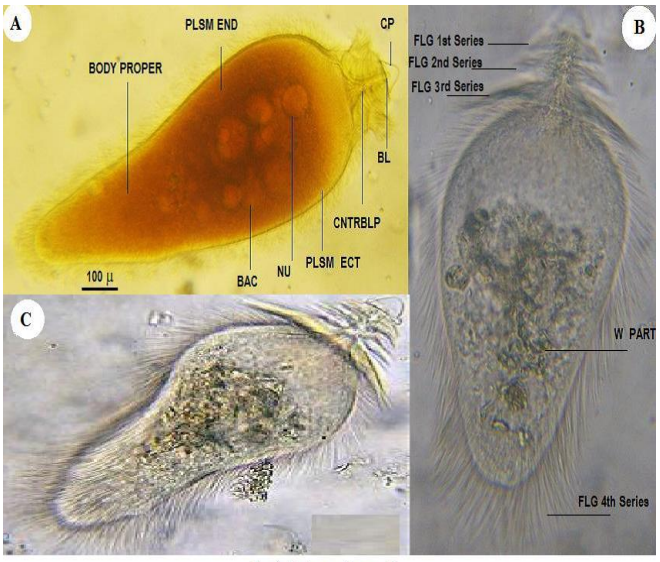
***Holomastigotoides hartmanni*:**
Diagnosis: Body pear-shaped with elongated and slightly pointed anterior part; most of the body covered with periplast which helps in permanency of body shape; posterior part of the body without periplast, no spiral bands are present; steriocilia are attached; the steriocilia are different from cilia because of their mode of attachment and are devoid of basal granules; flagella arise from basal granules that lies in the cytoplasm; nucleus lies near the anterior end; have 1to3 nucleoli (Fig. 4, Table 2 & 3).

GENUS

***Pseudotrichonympha*:** The genus *Pseudotrichonympha* is described by Grassii in 1917 for the first time as *Trichonympha*, then as *Pseudotrichonympha* by de Mello in 1927, Saleem in 1952 and Kudo in 1966.

***Diagnosis*:** spindle shaped body divided into two distinct parts; bell and body proper; bell at the anterior end of the centroblepharoplast is barrel-shaped and covered by a cap; spiral rows of flagella present on the whole body; cytoplasm divided into ectoplasm and endoplasm.

Pseudotrichonympha grassii:
Diagnosis: Spindle-shaped body; divided into head, neck and body proper; flagella arranged in the form of dextral spiral rows completely cover the body; head composed of a bell covered by a transparent hyaline cap; upper margin of the cap lies close to the bell and lower margin folded and devoid of flagella; neck also called the centroblepharoplast, tube like anteriorly and has varying thickness; centroblepharoplast composed of basal granules lying close to each other, which appear to constitute a single structure; neck flagella long, active, in the form of three series and perform jerky movements; flagella of Series 1 arise from the bell; their length increase from top to bottom and showed strong jerky movements. Flagella that root from the centroblepharoplast are referred to as *flagella of series 2* and they are longer than the flagella of series 1; Flagella of series 3 arise from the posterior region of the neck, are larger nucleus than the other two series and perform very active jerky movements during locomotion; round or oval in shape; has two nucleoli; chromatin material is evenly distributed or scattered randomly; nucleus has no definite position; it can be at the anterior end, the middle part, or the posterior end of the body (Fig. 5, Table 2 & 3).



Pseudotrichonympha grassii

Figure 5. Different microscopic views of *Pseudotrichonympha grassii* at 100X; CP (Cap); BL (Bell.); CNTRBLP (Centroblepharoplast); PLSM ECT (Ectoplasm); PLSM END (Endoplasm); W PART (Wood particles); NU (Nucleus); BAC (Bacteroidales); FLG (Flagella).

Table 4. Accession number of *Pseudotrichonympha grassii* from NCBI used for constructing a phylogenetic tree.

Taxon		NCBI Gene Bank accession	Country of origin
In group	*Pseudotrichonympha grassii*	JX649947	Pakistan
	Pseudotrichonympha grassii	AB262511	Japan
	Pseudotrichonympha grassii	AB262486	China
	Pseudotrichonympha grassii	AB032211	Japan
	Pseudotrichonympha sp.	AB262487	Malaysia
	Pseudotrichonympha sp.	AB262488	Laos
	Pseudotrichonympha sp.	AB262491	Brazil
	Pseudotrichonympha hertwigi	HQ683706	Canada
	Pseudotrichonympha paulistana	HQ683705	Canada
Out group	*Barbulanympha ufalula*	AB443595	Japan

***PCR amplification of SSU rRNA*:** Two sets of primers (Table 1) were used for amplification of the SSU rRNA gene. The amplification was confirmed by performing gel electrophoresis assay and the results revealed a DNA band of the expected size of 1.4 KB (Fig. 6).

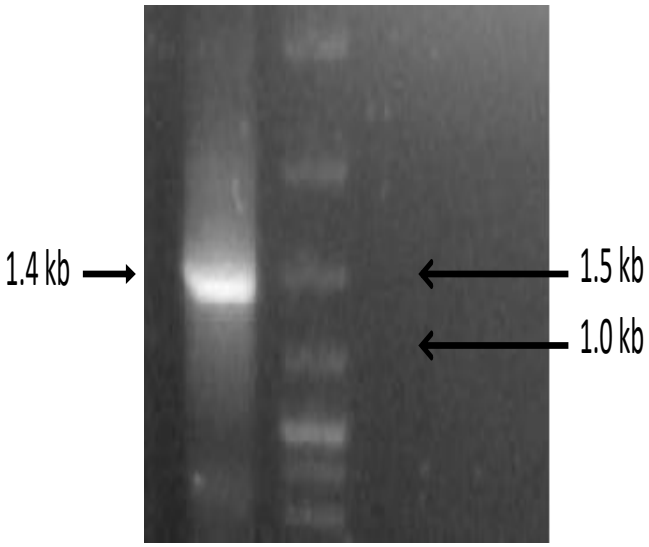


Figure 6. PCR amplified product of SSU rRNA of *P. grassii*.

***Phylogenetic analysis of P. grassii*:** BLAST analysis of the DNA sequence of the cloned PCR product performed on NCBI's website (http://blast.ncbi.nlm.nih.gov/) confirmed that the PCR product was *P. grassii* SSU rRNA gene. The phylogenetic tree shows that *P. grassii* isolated from Pakistan closely relates to the *P. grassii* isolated from Japan. Bayesian probability together with bootstrap values for Niebuhr Joining, Parsimony and Maximum Likelihood analysis strongly supported our phylogenetic tree and its clades (Fig. 7, Table 4). The accession JX649947 was obtained after the submission of sequences in Gene Bank.

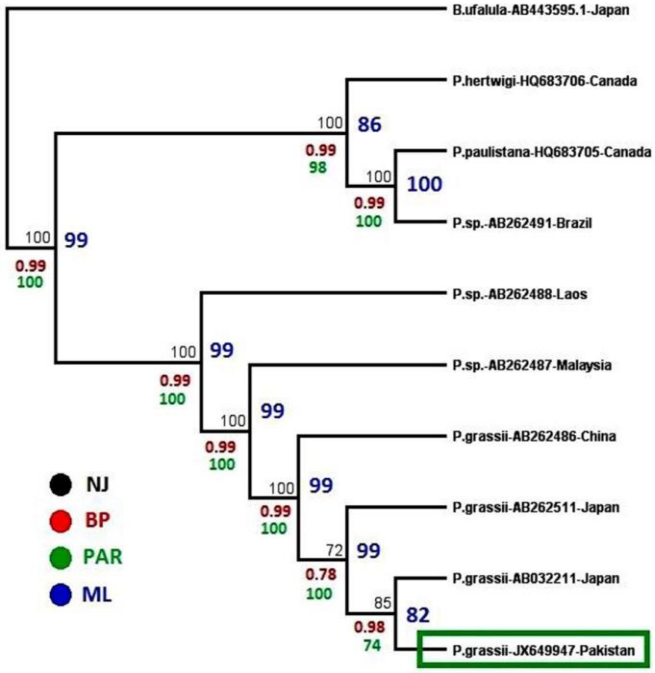


Figure 7. Molecular phylogenetic tree of *P. grassii* (NJ, Neighbor Joining; BP, Bayesian Probability; PAR, Parsimony; ML, Maximum Likelihood; for the details of taxa used.

DISCUSSION

In the present studies, the nine species of flagellates belonging to three genera viz, *Holomastigotes* (*H. campanula, H. annandalei* and *H. metchnikowi*), *Holomastigotoides* (*H. hemigynum, H. hartmanni, H. kempi, H. koidzumi* and *H. metchnikowi)* and *Pseudo trichonympha* (*P. grassii*) were identified from *H. indicola* morphologically. In addition, of *P. grassii* was identified on a molecular basis analyzed phylogenetically. Studies on the relative abundance of some species of protists in the hindgut of *H. indicola* demonstrated that *P. grassii* is the largest and the most abundant species found in *H. indicola*. The comparison of our *P. grassii* isolates

with the *P. grassii* isolate in other parts of the world, the results of our molecular phylogenetics are in agreement with those of Noda *et al.* (2005). Evolutionary tree (Fig. 7) depicts that Pakistani *P. grassii* isolate was closely related to the Japanese *P. grassii* isolate and falls in the Asian clade.

In the termite hindgut *P. grassii* is found replete with wood particles it is suggested that wood digestion largely depends upon this comparatively large flagellate. The *P. grassii* digests lignocellulose in the wood which is the major component of cellulose and provide digested food material to the termites. Thus *P. grassii* may alone be sufficient to fulfill the entire nutritional needs of the termite and dominates over the significance of other flagellate species. Therefore, antiprotozoal drugs against *P. grassii* may prove a valuable tool for termite eradication. *Pseudotrichonympha* occurs almost exclusively in Rhinotermitidae except for the genus *Reticulitermes* closely related to *Heterotermes* and *Coptotermes* (Inward *et al.,* 2007: Lo *et al.,* 2004). It is the only parabasalian symbiont identified in the genera *Termitogeton* and *Parrhinotermes* of termites (Kitade and Matsumoto, 1998). The protozoan fauna has a close relationship with their termite host and are transferred to their next host within the colony by protodeal trophallaxis (Inoue *et al.,* 2000) and from the mother colony to newly established colony by allates. Different termite genera possess different combinations of the gut protozoa. Even at a given location similarity in symbiotic fauna found in different termite genera is rarely observed (Kitade, 2004). There is a report that two unrelated genera *Reticulitermes* and *Hodotermopsis* have similar protists fauna (Kitade and Matsumoto, 1998). This unusual observation has been explained by Noda *et al.* (2007), which says that the intestinal fauna in *Reticulitermes* might have got replaced with those from close relatives or ancestors of *Hodotermopsis* through horizontal transfer. Saldarriaga *et al.* (2002) suggested if this hypothesis was true then some species of *Reticulitermes* might have in them *Pseudotrichonympha* spp. or any protozoan species that are common to other Rhinotermitids.

***Conclusion*:** The endomicrobes found in the hindgut of termites play a major role in cellulose digestion and manufacture cellulases that break down cellulose into acetate, propionate and butyrate. On the other hand, with the aid of these endomicrobes such small insects cause millions of damages to woods and wooden materials. Thus, by reviewing morphology and characteristics of symbiotic flagellates residing in the hindgut of *H. indicola* it was proposed that cellular digestion of hemicelluloses is possible due to the symbiotic association of protozoan flagellates. The termites gut flagellates have a key role in the survival of termites. By using protozoacides instead of insecticides may be more environment friendly as the low concentration of protozoacides will be required for killing their symbiotic flagellates.

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