Extensive diversity of intestinal trichomonads of non-human primates

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SUMMARY

Despite the fact that the non-human primates are our closest relatives and represent a species-rich mammalian group, little is known about their intestinal protistan parasites/commensals. Particularly, the intestinal trichomonads represent a neglected part of the fauna of the primate digestive system. We have established 30 trichomonad strains isolated from feces of 11 primate species kept in 3 Czech zoos and performed an analysis of their SSU rDNA and ITS1-5·8S rDNA-ITS2. Our results showed that intestinal trichomonads are rather common among non-human primates. Molecular phylogenetic analysis showed that the strains are unexpectedly diversified, belonging to 8 or 9 distinct species. Interestingly, the vast majority of the strains from non-human primates belonged to the genus *Tetratrichomonas* while no member of this genus has been found in the human intestine so far. In addition, hominoid and non-hominoid primates differed in their intestinal trichomonads. Our results suggest that captive primates possibly may be infected by intestinal trichomonads of other vertebrates such as pigs, cattle, birds, tortoises and lizards.

Key words: trichomonads, Parabasalia, non-human primates, diversity, host specificity.

INTRODUCTION

Trichomonads (Parabasalia) are flagellated protists classified into the eukaryotic supergroup Excavata (Cavalier-Smith, 2002). Most of the approximately 450 described trichomonad species (Adl *et al.* 2007) are beneficial mutualists or commensals of termites and other insects, while only a minority has been described from vertebrate intestines. Some trichomonad species, e.g. *Trichomonas vaginalis, T. gallinae, Tritrichomonas foetus*, and *Histomonas meleagridis*, are important pathogens of various internal organs of humans and domestic animals (Honigberg, 1978*a*, *b*; McDougald and Reid, 1978). Besides the endobiotic trichomonads, a few free-living representatives exist as well (Cepicka *et al.* 2010; Yubuki *et al.* 2010). Despite their relatively low species richness outside the insect gut, trichomonads have been commonly recorded from many vertebrate species. However, true diversity of trichomonads in vertebrates is only poorly understood. Several trichomonad species have been recently newly reported even from humans and domestic animals, whose endobiotic protists have been intensively studied for a long time (Levy *et al.* 2003; Cepicka *et al.* 2005, 2006; Kutisova *et al.* 2005; Dufernez *et al.* 2007; Mantini *et al.* 2009). Besides humans, domestic animals and a few other vertebrates, the diversity of intestinal trichomonads has been largely understudied, a situation that surprisingly also applies for non-human primates.

The non-human primates are our closest relatives and represent a species-rich mammalian group, several of whose members are seriously endangered. Despite several recent studies (e.g., Levecke *et al.* 2009; Stensvold *et al.* 2009; Johnston *et al.* 2010; Pomajbíková *et al.* 2010), the diversity of parasite communities in primates remains neglected in several aspects including trichomonads. So far, 9 species of intestinal trichomonads have been identified in non-human primates (e.g. Deschiens, 1927; Hegner and Ratcliffe, 1927; Wenrich, 1944*a*; Flick, 1954; Abraham, 1961, 1962; Culbertson *et al.* 1986; Pindak and de Pindak, 1998; Stark *et al.* 2008). This number is comparable to that of intestinal trichomonad species known from the other mammal groups, such as rodents, ruminants or suids. Most of the species were, however, reported only once in a single study

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Table 1. List of trichomonad strains included in the study

(A, apes; P, prosimians; S, simians.)

Isolate	Host	Host common name	Zoo
GANG1	*Colobus angolensis*[S]	Angola colobus	Plzeň
GSIA1	*Hylobates syndactylus*[A]	siamang	Ostrava
GSIA2	*Hylobates syndactylus*[A]	siamang	Ostrava
GSIA3	*Hylobates syndactylus*[A]	siamang	Ostrava
GSIA4	*Hylobates syndactylus*[A]	siamang	Ostrava
JIMBO1	*Pan troglodytes*[A]	chimpanzee	Ostrava
KATA1	*Lemur catta*[P]	ring-tailed lemur	Plzeň
KATASAMEC	*Lemur catta*[P]	ring-tailed lemur	Plzeň
KOMBG1	*Otolemur garnettii*[P]	northern greater galago	Plzeň
KOSBE1	*Callithrix jacchus*[S]	common marmoset	Plzeň
MA3	*Macaca silenus*[S]	lion-tailed macaque	Ostrava
MA4	*Macaca silenus*[S]	lion-tailed macaque	Ostrava
MANI1	*Macaca nigra*[S]	crested macaque	Brno
MANI2	*Macaca nigra*[S]	crested macaque	Brno
MASP1	*Mandrillus sphinx*[S]	mandrill	Brno
PAN1	*Pan troglodytes*[A]	chimpanzee	Plzeň
PAN2	*Pan troglodytes*[A]	chimpanzee	Plzeň
PAN3	*Pan troglodytes*[A]	chimpanzee	Plzeň
PAN4	*Pan troglodytes*[A]	chimpanzee	Plzeň
PAN5	*Pan troglodytes*[A]	chimpanzee	Plzeň
PAN6	*Pan troglodytes*[A]	chimpanzee	Ostrava
PAN7	*Pan troglodytes*[A]	chimpanzee	Ostrava
PAN8	*Pan troglodytes*[A]	chimpanzee	Ostrava
PAN9	*Pan troglodytes*[A]	chimpanzee	Ostrava
PAN10	*Pan troglodytes*[A]	chimpanzee	Ostrava
PAN11	*Pan troglodytes*[A]	chimpanzee	Ostrava
SENT1	*Semnopithecus entellus*[S]	Hanuman langur	Ostrava
SENT3	*Semnopithecus entellus*[S]	Hanuman langur	Ostrava
SENT4	*Semnopithecus entellus*[S]	Hanuman langur	Ostrava
VARI1	*Varecia variegata*[P]	ruffed lemur	Plzeň

and never have been observed again. The morphological descriptions were usually inadequate and they cannot be considered in taxonomical studies. In addition, almost no sequence data of any trichomonad from non-human primates are currently available. The lack of sequence data is especially unsatisfactory as it was recently shown that SSU rDNA and the ITS region could be used as a suitable barcode for trichomonads (e.g. Cepicka *et al*. 2006; Dufernez *et al*. 2007).

Recently, primates have re-attracted attention of parasitologists and thorough studies of some of their intestinal protists have been carried out (Levecke *et al*. 2009; Stensvold *et al*. 2009; Johnston *et al*. 2010; Pomajbíková *et al*. 2010). This report describes the first molecular-phylogenetic study to investigate the diversity of intestinal trichomonads of non-human primates.

MATERIALS AND METHODS

Organisms

Thirty-five fresh fecal samples of 12 primate species were collected in 3 zoos, namely those in Brno, Pilsen and Ostrava (Czech Republic) from March to July 2005. The samples were immediately inoculated into Dobell and Leidlaw's biphasic medium (Dobell and Leidlaw, 1926) and were cultivated at 37 °C after transport to the laboratory. The isolates were maintained in xenic cultures in this medium by serial transfer every 2nd or 3rd day. In total, 30 stable trichomonad strains obtained from 11 primate species have been established (see Table 1). The remaining 5 samples were negative for trichomonads: 2 from olive baboons (*Papio anubis*) from the Brno Zoo, 1 from a chimpanzee (*Pan troglodytes*) from the Ostrava Zoo, 1 from a chimpanzee from the Brno Zoo and 1 from a Hanuman langur (*Semnopithecus entellus*) from the Ostrava Zoo. Strains GANG1, GSIA1, KATA1, KATASAMEC, KOMBG1, KOSBE1, MA3, MANI1, MANI2, MASP1, PAN3, SENT1, SENT2, and VARI1 have been deposited in the culture collection of the Department of Parasitology of Charles University in Prague, Czech Republic.

DNA extraction, amplification, cloning and sequencing

Genomic DNA was isolated from roughly the fourth passage of cultures using the DNeasy Blood & Tissue Kit (Qiagen). Primers 16Sl (TACTTGGTTGATCCTGCC; Tachezy *et al*. 2002) and 16SRR (TCACCTACCGTTACCTTG; Cepicka *et al*. 2005) were

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used to amplify SSU rDNA. Primers ITSF (TTCAGTTCAGCGGGTCTTCC) and ITSR (GTAGGTGAACCTGCCGTTGG) (Cepicka *et al*. 2005) were used to amplify ITS1-5.8S rDNA-ITS2. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and were directly sequenced on the ABI Prism 3100-*Avant* Genetic Analyzer (Applied Biosystems). The ITS region of the isolate GSIA1 was cloned into the pGEM®-T EASY vector (Promega). Eight clones were sequenced. The newly obtained sequences have been deposited in GenBank under Accession numbers HQ149966 – HQ150005.

Phylogenetic analyses

Pre-emptive phylogenetic analysis of obtained SSU rDNA sequences placed all but 11 of the newly obtained sequences to the *Tetratrichomonas* group A, as defined by Cepicka *et al*. (2006). All these isolates also contained the 2 SSU rDNA insertions typical for group A (Cepicka *et al*. 2006). Because much of the sequence variability of tetratrichomonad group A was lost during the removal of hypervariable positions from the alignment representing broad parabasalid diversity, SSU rDNA sequences were used in 2 separate analyses. The first data set consisted of 47 SSU rDNA sequences representing all main parabasalid lineages and 11 of the new sequences which did not belong to the tetratrichomonad group A. The second data set consisted of 25 published SSU rDNA sequences of the tetratrichomonad group A, including the LP isolate, 19 newly obtained sequences placed in group A, and 3 non-group-A *Tetratrichomonas* sequences used as outgroups. In addition to the 2 data sets, a third data set containing ITS1-5.8S rDNA-ITS2 sequences of tetratrichomonad group A (40 obtained from GenBank and 10 new ones) was created. Sequences from each data set were aligned using the MAFFT method (Katoh *et al*. 2002) with the help of the MAFFT 6 server (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) using the G-INS-i algorithm at default settings. The resulting alignments were manually edited in BioEdit 7.0.9.0 (Hall, 1999). The lengths of the final alignments were 1323 (first data set), 1538 (second data set), and 345 (third data set) characters. The alignments are available from the corresponding author upon request.

Phylogenetic trees were constructed by maximum parsimony (MP), Fitch-Margoliash distance method with maximum likelihood distances (MLdist), maximum likelihood (ML), and Bayesian methods. The models of nucleotide substitution for ML and MLdist methods were chosen by a hierarchical nested likelihood ratio test implemented in Modeltest 3.7 (Posada and Crandall, 1998). The models were selected as follows: TrN + I + Γ for the first data set, GTR + I + Γ for the second data set and F81 + I + Γ for the third data set. MP and MLdist trees were constructed in PAUP* 4.0b10 (Swofford, 2002) by 10 replicates of heuristic search in which the starting tree was obtained by the stepwise addition procedure with a random order of taxon addition and swapped using the tree bisection and reconnection (TBR) algorithm. The trees were bootstrapped with 1000 replicates. ML trees were constructed in Phyml (Guindon and Gascuel, 2003) and were bootstrapped with 1000 replicates. Bayesian analyses were performed using MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). Base frequencies, rates for the 6 different types of substitution, the proportion of invariable sites and the shape parameter of the gamma correction for the rate heterogeneity (approximated by 4 discrete categories) were allowed to vary. A co-varion model was used to allow rate heterogeneity along the tree. The number of generations of Markov chain Monte Carlo was 2×10^6 for the first and third data set, and 3×10^6 for the second data set (until the average standard deviation of split frequencies was lower than 0·01); the trees were sampled every 100th generation. The first 5000 (first and third data set) or 7500 (second data set) trees were discarded as burn-in.

RESULTS

Trichomonads were present in approximately 85% in primocultures on the second day after the inoculation.

Figure 1 shows the phylogenetic tree of Parabasalia constructed from SSU rDNA sequences on the basis of the first data set. The tree topology was similar to the topology obtained by Cepicka *et al*. (2010). Classes Trichonymphea, Trichomitea, Cristamonadea, Spirotrichonymphea and Trichomitea formed robust clades with all the used methods. The class Tritrichomonadea formed an unsupported clade only in ML analysis (bootstrap value 39), whereas it was paraphyletic, having Cristamonadea as an inner branch, in the other analyses. The internal topology of Tritrichomonadea was not well resolved. The close relationship between Tritrichomonadea and Cristamonadea was recovered by all methods, although without strong support. The class Trichomonadea was always paraphyletic, having Trichonymphea as an inner branch. However, the position of Trichonymphea was not well supported by any method. The strain KOMBG1 formed a well-supported clade with *Hypotrichomonas acosta*. The strain KATA1 robustly clustered with *Trichomitus batrachorum*. Sequences of strains GANG1, KOSBE1 and VARI1 were identical with that of *Pentatrichomonas hominis*. The strain KATASAMEC was placed to the *Tetratrichomonas gallinarum* species complex and occupied a sister position of the GPO strain. Sequences of the MA3, MA4, MANI1,

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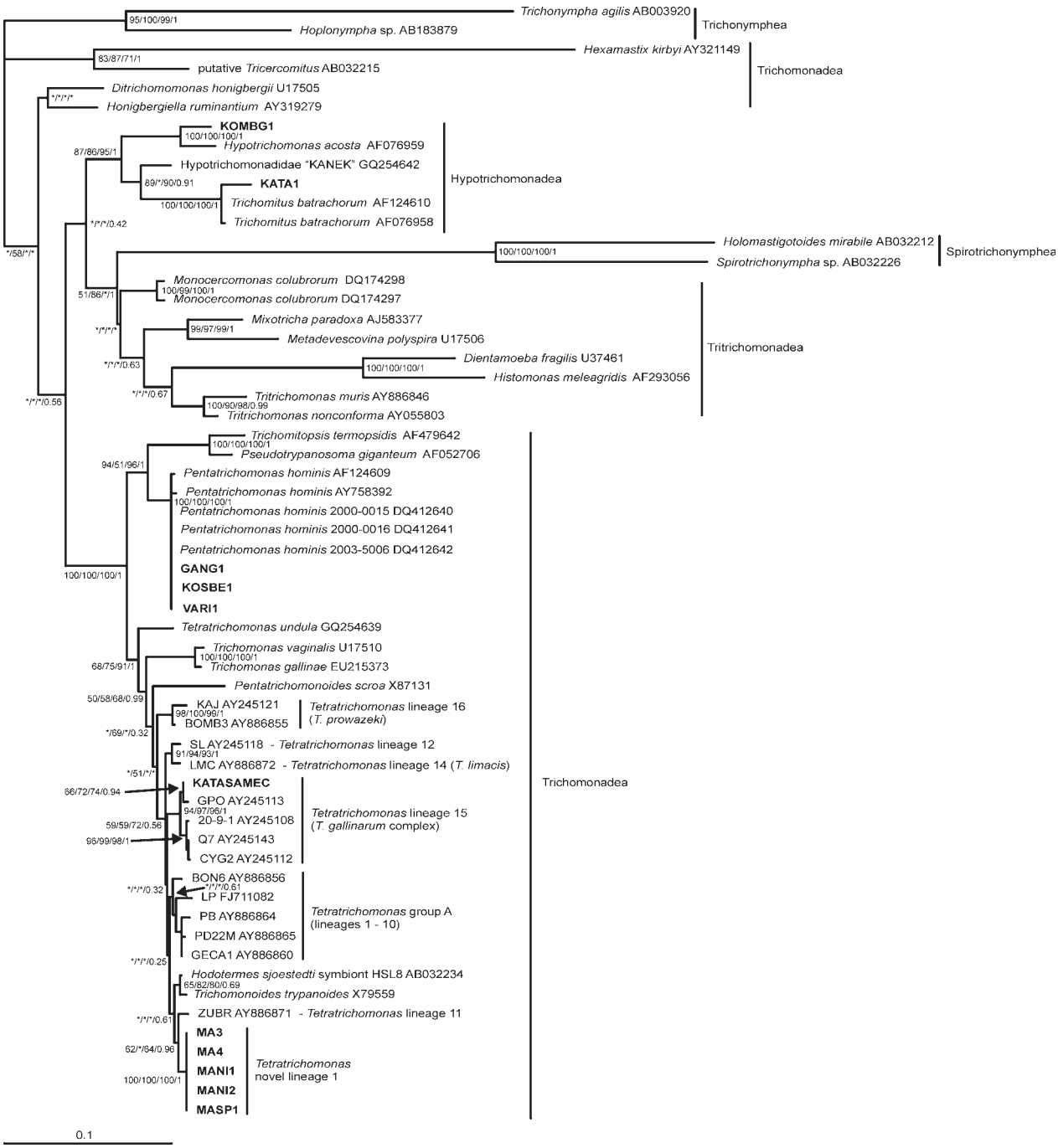

Fig. 1. Phylogenetic tree of Parabasalia based on SSU rDNA. The tree was constructed from the first data set by ML method under the TrN + I + Γ substitution model and is unrooted. Values at the nodes represent statistical support (MP bootstrap value/MLdist bootstrap value/ML bootstrap value/Bayesian posterior probability). Asterisks indicate nodes with bootstrap support under 50%. The newly obtained sequences are in bold.

MANI2, and MASP1 strains were identical and formed a novel tetratrichomonad lineage (here called novel *Tetratrichomonas* lineage 1) that clustered, albeit weakly, with the ZUBR strain (*Tetratrichomonas* lineage 11). Tetratrichomonad group A was recovered by all methods with low support.

All remaining strains of trichomonads from primates belonged to *Tetratrichomonas* group A. The LP strain from human (Mantini *et al*. 2009) was regarded as a member of group A on the basis of 2 insertions in the SSU rDNA sequence that are typical for the group (Cepicka *et al*. 2006). Figure 2 shows the phylogenetic tree of *Tetratrichomonas* group A constructed from SSU rDNA sequences on the basis of the second data set. The tetratrichomonad lineages defined by Cepicka *et al*. (2006) were robustly recovered. However, relationships between particular tetratrichomonad lineages were generally unsupported, correspondingly to results of previous studies (Cepicka *et al*. 2006; Dufernez *et al*. 2007; Mantini *et al*. 2009). The trichomonad LP formed a basal branch of tetratrichomonad group A. The

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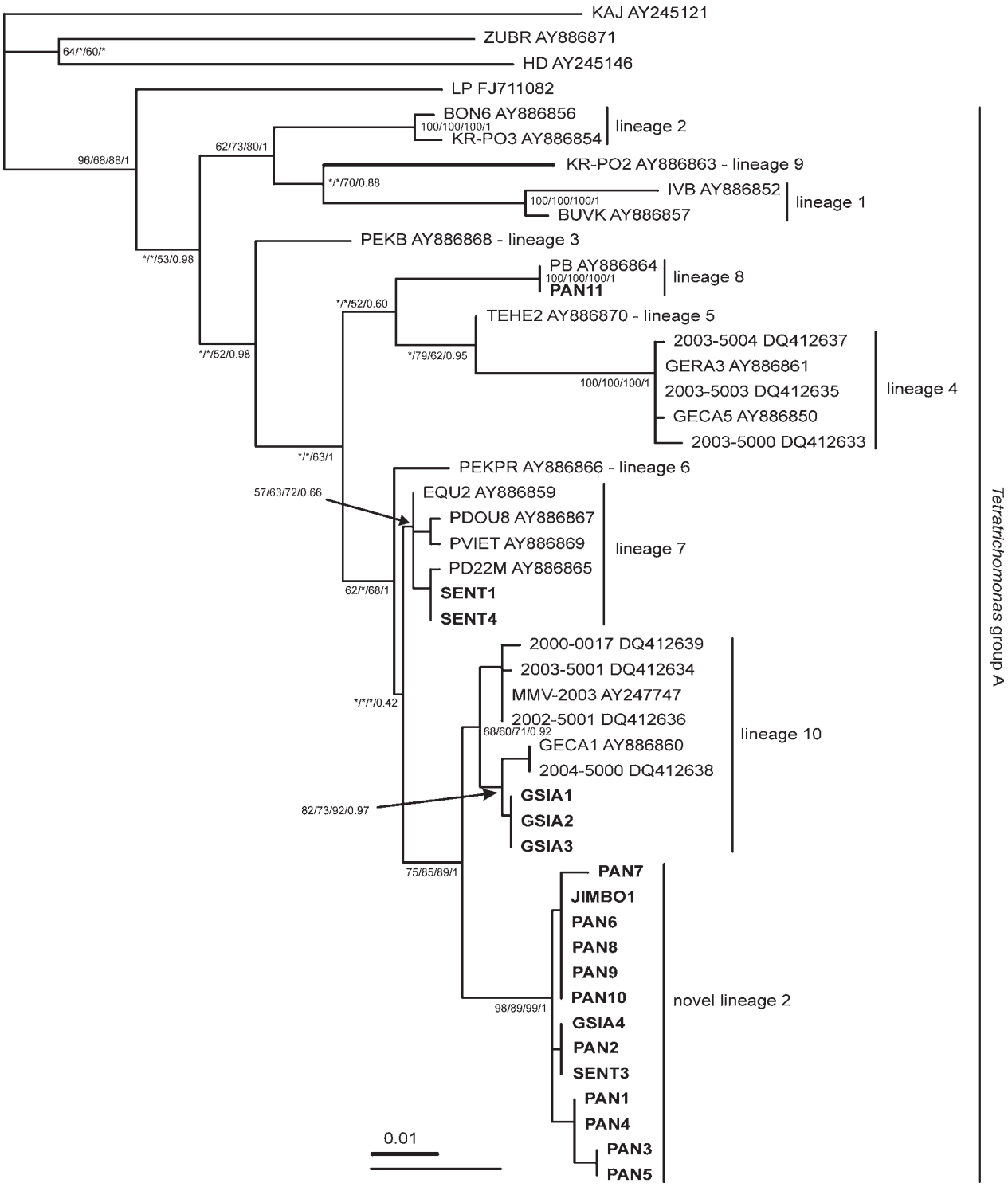

Fig. 2. Phylogenetic tree of *Tetratrichomonas* group A based on SSU rDNA. The tree was constructed from the second data set by ML method under the GTR + I + Γ substitution model and was rooted with *Tetratrichomonas* KAJ, ZUBR and HD. Values at the nodes indicate statistical support (MP bootstrap value/MLdist bootstrap value/ML bootstrap value/Bayesian posterior probability). Asterisks indicate nodes with bootstrap support under 50%. *Tetratrichomonas* lineages as defined by Cepicka *et al*. (2006) are indicated. The branch length of *Tetratrichomonas* sp. KR-PO2 was reduced by one half. The newly obtained sequences are in bold.

sequence of the PAN11 strain was identical with that of the *Tetratrichomonas* sp. PB strain (lineage 8). SENT1 and SENT4 strains had identical SSU rDNA sequences and belonged to *Tetratrichomonas* lineage 7. Strains GSIA1, GSIA2, and GSIA3 had identical sequences and clustered within *Tetratrichomonas* group 10. The sequence of the trichomonad MMV-2003 obtained from GenBank (Accession number AY247747) from a black crested gibbon (*Nomascus concolor*) was placed within lineage

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10 as well. The remaining 13 strains from non-human primates formed a novel tetratrichomonad lineage (here called the novel *Tetratrichomonas* lineage 2), which was closely related to lineage 10. The mean genetic distance (p-distance) between lineage 10 and the new lineage was 1·0%.

Figure 3 shows the phylogenetic tree of *Tetratrichomonas* group A constructed from ITS1-5.8S rRNA-ITS2 on the basis of the third data set. All *Tetratrichomonas* lineages appeared monophyletic and well supported. However, relationships between the particular lineages remained mostly unresolved, as in a previous study (Cepicka *et al*. 2006). Contrary to the SSU rDNA analysis, the trichomonad LP formed a robust clade with lineages 4 and 5. The sister branch of this clade was formed by the KR-PO2 strain (lineage 9), although with low support. The phylogenetic position of the newly obtained strains was in agreement with the SSU rDNA analysis. SENT1 and SENT4 strains with identical sequences were placed within lineage 7. The PAN11 strain was in a sister position to the PB strain. The ITS region of the GSIA1 strain was subcloned because it was impossible to directly sequence the PCR product. Sequences of 7 clones were almost identical, differing in at most 2 positions. However, their consensual sequence differed from the sequence of the clone 1_10 in 6 substitutions and 1 indel, which indicated that either intragenomic polymorphism or 2 different, though closely related, organisms were present within the GSIA1 strain. Both of the sequences branched within lineage 10. Strains PAN2, PAN3, PAN6, PAN9, and JIMBO1 formed a robust clade closely related to lineage 10. The mean genetic distance between lineage 10 and the new clade was 3·8%.

DISCUSSION

Although non-human primates are our closest relatives, investigation of their intestinal parasites has been neglected for decades. Most of the studies focused on their intestinal trichomonads were published before the introduction of molecular phylogenetics into protistology, and were thus limited to light microscopy (e.g. Deschiens, 1927; Wenrich, 1944*a*; Flick, 1954; Abraham, 1961, 1962; Reardon and Rininger, 1968; Culbertson *et al*. 1986; Pindak and de Pindak, 1998). Unfortunately, the morphological descriptions were mostly inadequate, too brief or confusing. Although intestinal parasites of non-human primates have attracted more attention during the last years, new reports pertaining to intestinal trichomonads almost ceased. Only 4 studies of intestinal trichomonads of non-human primates were published recently (Lilly *et al*. 2002; Carmona *et al*. 2005; Stark *et al*. 2008; Lankester *et al*. 2010), the 2 former being almost uninformative. Such a paucity of data on this group contrasts with literature addressing the diversity of *Entamoeba* (Suzuki *et al*. 2008; Tachibana *et al*. 2009), *Blastocystis* (Stensvold *et al*. 2009; Parkar *et al*. 2010), *Giardia* (Levecke *et al*. 2009; Johnston *et al*. 2010), entodiniomorphid ciliates (Pomajbíková *et al*. 2010; Tokiwa *et al*. 2010) or nematodes (e.g. Cutillas *et al*. 2009; Hasegawa *et al*. 2010; Krief *et al*. 2010). The reason for such a discrepancy is most likely connected to a methodological bias. At present, coproscopical methods are mostly employed to study the intestinal parasites of non-human primates. They are optimized for the detection of cysts of protists and eggs of helminths (Greiner and McIntosh, 2009). However, the ability to form true cysts was demonstrated only for a few trichomonad species (Hampl *et al*. 2007). The vast majority of trichomonads produce only trophozoites that remain undetected by the coprological methods. On the other hand, a PCR-based approach, which has been used for trichomonads from various other host taxa, has been employed only once in the case of non-human primates (Stark *et al*. 2008). In the present study, we used this promising approach to study the diversity of cultivated trichomonad strains obtained from the feces of various non-human primate species.

Our results show that intestinal trichomonads are rather common in various species of non-human primates. To exclude possible cultivation bias, i.e. the loss of uncultivable trichomonad species during early passages, we created polyxenic cultures in the Dobell and Leidlaw's (1926) biphasic medium. Our previous experience showed that most trichomonad species are able to survive at least a few passages under such conditions. To detect trichomonads, the cultures were microscopically controlled the second day after the inoculation of feces into the medium. We are therefore convinced that the results correspond to the actual occurrence of these trichomonads among examined individuals.

According to their distribution in phylogenetic trees, the studied trichomonad isolates from non-human primates belong to 8 or 9 distinct species. As far as we know, 9 trichomonad species have been reported from the intestine of primates, including humans (Deschiens, 1927; Cleveland, 1928; Wenrich, 1944*a*; Flick, 1954; Abraham, 1961, 1962; Culbertson *et al*. 1986; Pindak and de Pindak, 1998; Stark *et al*. 2008). However, all but 1 of these species either are clearly distinct from our isolates or were inadequately described and thus cannot be considered as valid in taxonomic studies. The morphological evaluation of the presented material is beyond the scope of this study and will be published elsewhere.

The KOMBG1 strain from a northern greater galago (*Otolemur garnettii*) clustered with *Hypotrichomonas acosta* from a snake, and probably represents an undescribed species within this genus. The genus *Hypotrichomonas* belongs to a small

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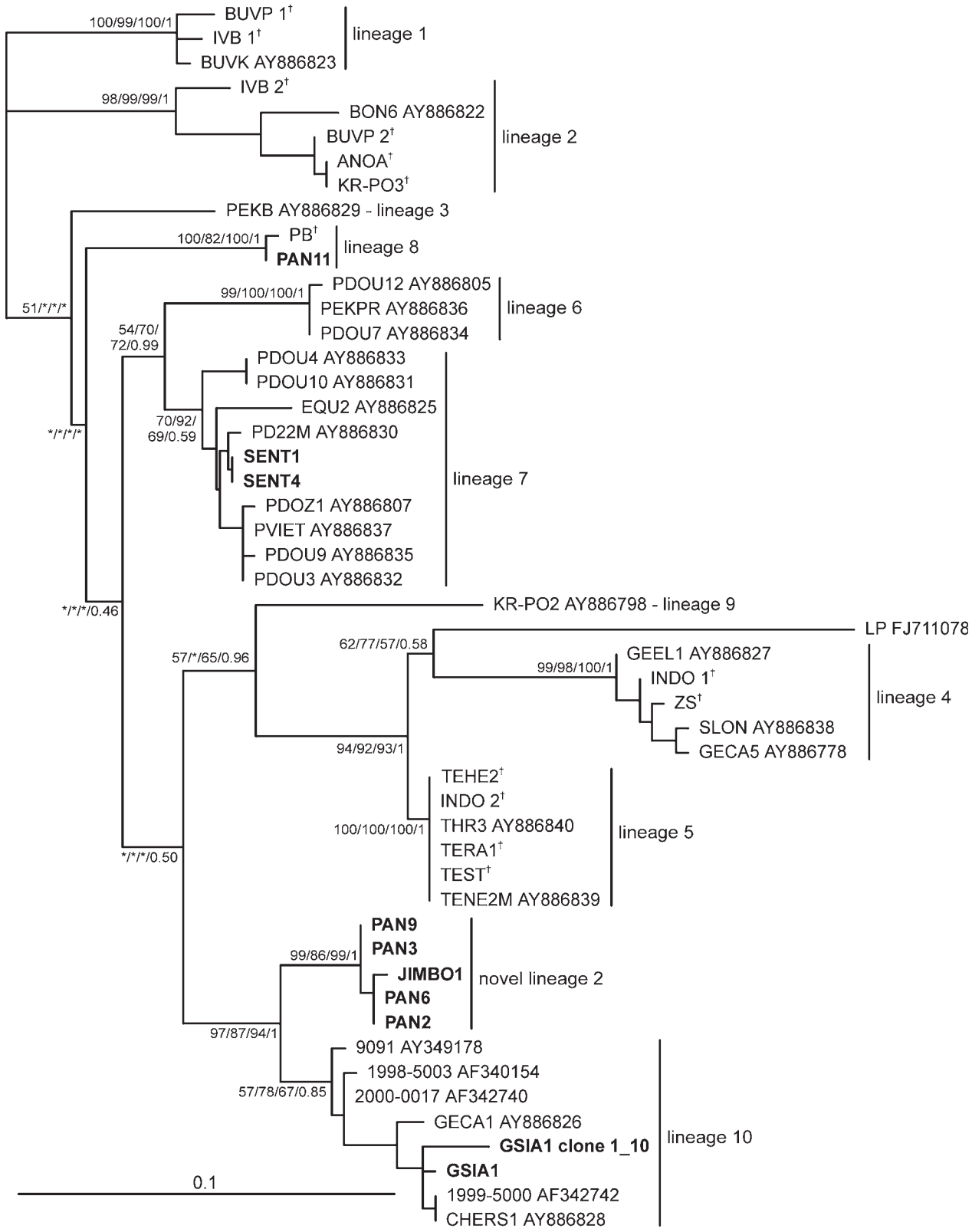

Fig. 3. Phylogenetic tree of tetratrichomonad group A based on ITS1-5.8S rDNA-ITS2. The tree was constructed from the third data set by ML method under the F81 + I + Γ substitution model and is unrooted. Values at the nodes indicate statistical support (MP bootstrap value/MLdist bootstrap value/ML bootstrap value/Bayesian posterior probability). Asterisks indicate nodes with bootstrap support under 50%. *Tetratrichomonas* lineages as defined by Cepicka *et al*. (2006) are indicated. The newly obtained sequences are in bold.

though evolutionarily distinct parabasalid lineage called Hypotrichomonadea (Cepicka *et al*. 2010). *H*. *acosta*, which is the only member of the *Hypotrichomonas* whose cell structure has been examined by electron microscopy, possesses an interestingly reduced costa (a striated fibre that

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underlies the undulating membrane in some trichomonads). As we have successfully established a stable culture of a second *Hypotrichomonas* species, a future TEM study of the KOMBG1 isolate is desirable.

The KATA1 strain from a ring-tailed lemur (*Lemur catta*) is closely related to *Trichomitus batrachorum*, suggesting that it belongs to the genus *Trichomitus*, the second genus of Hypotrichomonadea. So far, 2 *Trichomitus* species have been reported from primates, though each of them only once. *T. wenyoni* was originally described from laboratory rodents (Wenrich and Nie, 1949) and has been also reported from a rhesus macaque (Flick, 1954). However, no picture or drawing that would corroborate the observation was included in the latter study. We thus consider the presence of *T. wenyoni* in primates as uncertain (see also Culbertson *et al.* 1986). The second species of the genus *Trichomitus* from primates, *T. fecalis*, was described from a single human subject (Cleveland, 1928). It is morphologically very similar to *T. batrachorum* from amphibians, reptiles and leeches (Wenrich, 1944*b*). It is also infectious for tadpoles and frogs (Cleveland, 1928), suggesting that the two species may actually be conspecific. As the diversity of the genus *Trichomitus* is considerably undersampled in molecular-phylogenetic studies (SSU rDNA sequences of only 2 isolates of *T. batrachorum* are currently available from the whole genus), we cannot decide to which *Trichomitus* species the KATA1 isolate belongs solely on the basis of the sequence data.

Pentatrichomonas hominis has a rather broad host range and has been repeatedly found in many mammals including humans and non-human primates (Wenrich, 1944*a*; Flick, 1954; Reardon and Rininger, 1968). Our strains GANG1 from an Angola colobus (*Colobus angolensis*), VARI1 from a ruffed lemur (*Varecia variegata*) and KOSBE1 from a common marmoset (*Callithrix jacchus*) have identical or almost identical SSU rDNA sequences with *P. hominis* isolated from cattle and humans. We thus confirmed the presence of this wide-host-range species in non-human primates.

The rest of the isolates belonged to the species-rich genus *Tetratrichomonas*. The second strain from a ring-tailed lemur, KATASAMEC, surprisingly branched within *Tetratrichomonas gallinarum*. This trichomonad, once thought to be a specific intestinal parasite of birds, has been recently found also in human oral cavity, bronchi and sputum (Kutisova *et al.* 2005). It was also shown that *T. gallinarum* represents, in fact, an intricate complex of multiple species, some of which display considerable host specificity (Cepicka *et al.* 2005). The KATASAMEC strain formed an independent lineage, closely related, although without good bootstrap support, to the GPO strain isolated from chicken (Cepicka *et al.* 2005). Since the diversity of the basal members of the *T. gallinarum* species complex has not yet been satisfactorily investigated, it is currently impossible to determine whether the KATASAMEC strain represents an independent species.

Strains MA3, MA4, MANI1, MANI2 and MASP1 formed a novel *Tetratrichomonas* lineage. The isolates had identical sequences, originated from 2 different zoos and were obtained from 3 cercopithecine species (lion-tailed macaque – *Macaca silenus*, crested macaques – *Macaca nigra* and mandrill – *Mandrillus sphinx*). Since no close relative of the novel lineage is known, it might represent a new species. However, a morphological study should be performed before its formal description.

The remaining 19 strains belonged to tetratrichomonad group A as defined by Cepicka *et al.* (2006). Group A contains several *Tetratrichomonas* lineages that have been isolated from various mammals and tortoises and have been shown to represent distinct, though mostly undescribed, species (Cepicka *et al.* 2006). Our analyses also have clearly shown that the LP strain, which has been isolated from human pleural empyema (Mantini *et al.* 2009), belongs to *Tetratrichomonas* group A as well. The LP strain thus represents the second species from the respiratory system of humans in addition to *T. gallinarum*. The isolates from non-human primates occupied 4 different positions within tetratrichomonad group A. Strains SENT1 and SENT4 from Hanuman langurs (*Semnopithecus entellus*) were placed within lineage 7, which corresponds to *Tetratrichomonas buttreyi* and has been previously observed in wild and domestic pigs and a horse. The PAN11 strain from a common chimpanzee (*Pan troglodytes*) belongs to lineage 8, whose only previous representative, the PB strain, was isolated from a desert warthog kept in a zoo (Cepicka *et al.* 2006).

Three strains from siamangs (*Symphalangus syndactylus*) and the trichomonad MMV-2003 (GenBank Accession number AY247747) obtained from a black crested gibbon branched within lineage 10, which represents so far undescribed *Tetratrichomonas* species from cattle and tortoises (Cepicka *et al.* 2006). In addition, all but 1 isolate from chimpanzees together with the GSIA4 isolate from a siamang and the SENT3 isolate from a Hanuman langur formed the novel *Tetratrichomonas* lineage 2, closely related to lineage 10. It is currently impossible to decide whether lineage 10 and the new tetratrichomonad lineage are conspecific and a further morphological or infection study is necessary to satisfactorily address this question.

To assess the host specificity of any symbiotic organisms based on the data from captive animals is always complicated; trichomonads are no exception. Contacts with novel hosts, environments and ecological conditions as well as differences in food composition might distort the original trichomonad-host relationships. On the other hand, the

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transmission of intestinal non-cyst-forming trichomonads requires relatively intimate contact between hosts, which might preclude accidental transmissions. The distribution of our trichomonad strains in phylogenetic trees points to 3 interesting and unexpected aspects related to the host specificity of trichomonads.

(1) Some of the strains clustered within *Tetratrichomonas* lineages 7, 8, 10 and 15, which are dominated by trichomonads from non-primate hosts, i.e. pigs, cattle, tortoises, lizards, and birds (Cepicka *et al*. 2006). This observation means that at least some species of primates may be infected by intestinal trichomonads of these hosts and the host range of the 4 *Tetratrichomonas* lineages is wider than previously expected by Cepicka *et al*. (2006). A similar situation was also described in the case of *Blastocystis hominis* (Stensvold *et al*. 2009). Yet, at least 2 of the novel *Tetratrichomonas* lineages may turn out to be specific for primates. Host specificity of most intestinal trichomonads is unclear. Most species are assumed to be confined to a relatively narrow group of hosts, e.g. *Dientamoeba fragilis* to humans and gorillas (Stark *et al*. 2008), and *Tetratrichomonas limacis* to snails (Cepicka *et al*. 2006). On the other hand, at least one generalist species, *Pentatrichomonas hominis* from a wide range of warm-blooded hosts including humans, is known as well (see Honigberg, 1978*b*). Moreover, several trichomonad species parasitizing various non-primate hosts have been detected in the respiratory tract of humans (e.g. Cepicka *et al*. 2005; Kutisova *et al*. 2005; Duboucher *et al*. 2006). Our data indicate that many trichomonad species may be, in fact, less specific than currently assumed.

(2) Trichomonads of hominoid and non-hominoid primates occupied strikingly different positions in our phylogenetic trees. All strains from hominoids, i.e. chimpanzees, siamangs and a black crested gibbon (GenBank sequence AY247747) belonged exclusively to *Tetratrichomonas* group A. On the contrary, the strains from non-hominoid primates occupied diverse positions and only 2 strains, SENT1 and SENT4 from Hanuman langur belonged to *Tetratrichomonas* group A.

(3) The closely related humans and chimpanzees possess different intestinal trichomonads. Three trichomonad species have been reported from the human intestine, namely *Pentatrichomonas hominis, Trichomitus fecalis* and *Dientamoeba fragilis*. Only *P. hominis* has been detected also in chimpanzees (Myers and Kuntz, 1972). In contrast, all but 1 strain that we isolated from captive chimpanzees belonged to the novel *Tetratrichomonas* lineage 2. Although 2 different *Tetratrichomonas* species (unrelated to our strains from primates) have been retrieved from the human respiratory tract (Cepicka *et al*. 2005; Kutisova *et al*. 2005; Mantini *et al*. 2009), none has been found in the human intestine.

Most intestinal trichomonads are considered to be harmless commensals in mammalian hosts, but only little data are available to support this assumption. As demonstrated very recently, humans and chimpanzees share not only agents of malaria (Prugnolle *et al*. 2009), but also a broad spectrum of gastrointestinal parasites (Cutillas *et al*. 2009; Hasegawa *et al*. 2010; Krief *et al*. 2010). On the other hand, the community of their intestinal commensal/mutualistic protists differs substantially, which was demonstrated particularly for ciliates (Kortland: comment in Stahl *et al*. 1984; Pomajbíková *et al*. 2010; Tokiwa *et al*. 2010). An appealing scenario is that these differences were brought about by significant changes in the human diet during evolution. The hominid diet before the introduction of fire was more similar to that of great apes, characterized by a high fibre content (Stahl *et al*. 1984; Wrangham and Conklin-Brittain, 2003; Carmody and Wrangham, 2009) and both the ciliates and trichomonads seems to be to some extent influenced by diet composition (Ratcliffe, 1928; Pomajbíková *et al*. 2010). We might suggest that our ancestors harboured trichomonads similar to other hominoids, especially chimpanzees, as was hypothesized also for entodiniomorphid ciliates that are present in chimpanzees, but absent in humans (Kortland: comment in Stahl *et al*. 1984). However, to reveal the extent of the discrepancy of trichomonad species occurring in humans and chimpanzees, data from wild great apes are urgently needed.

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