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Cattle Pathogen *Tritrichomonas foetus* (Riedmüller, 1928) and Pig Commensal *Tritrichomonas suis* (Gruby & Delafond, 1843) Belong to the Same Species

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ABSTRACT. A number of reports suggest that the sexually transmitted pathogen of cattle, *Tritrichomonas foetus*, and a gastrointestinal commensal of pigs, *Tritrichomonas suis*, are very similar and may be co-specific. A conclusive review of the taxonomic and nomenclatural status of these species has not been presented so far. Toward this end, we reexamined and compared porcine and bovine trichomonads with regard to their morphology, pathogenic potential, and DNA polymorphism. Using light and electron microscopy, no distinguishing features between *T. foetus* and *T. suis* strains were found in size, general morphology, and karyomastigont structure. Both bovine and porcine trichomonads showed pathogenic potential in the subcutaneous mouse assays and did not separate into distinct groups according to strain virulence. Three DNA fingerprinting methods (i.e. RFLP, RAPD, and PCR-based analysis of variable-length DNA repeats) that produce species-specific DNA fragment patterns did not distinguish between the bovine and porcine strains. Sequencing of a variable 502-bp DNA fragment as well as comparison of 16S rRNA gene sequences did not reveal species-specific differences between the cattle and porcine strains. Therefore, we conclude that *T. foetus* and *T. suis* belong to the same species. To prevent confusion that may arise from *T. foetus–T. suis* synonymy, we propose to suppress the older name *suis* and maintain its accustomed junior synonym *foetus* as a *nomen protectum* for both cattle and porcine trichomonads. The case has been submitted to the International Commision on Zoological Nomenclature for ruling under its plenary power.

Key Words. Morphology, nomenclature, pathogenicity, RAPD, RFLP, taxonomy, *Tritrichomonas augusta*, *Tritrichomonas mobilensis*, *Tritrichomonas nonconforma*, 16S rRNA.

Tritrichomonas foetus is a pathogenic flagellate that causes a sexually transmitted invasion of the reproductive tract in cattle. In cows, mainly the uterus is infected, with transient infection of the cervix uteri and the vagina. In bulls, the preputial cavity, and less frequently the urethral orifice are infected. Clinical symptoms in females vary from mild vaginitis or endometritis to acute inflammation throughout the reproductive tract. In pregnancy, bovine trichomoniasis may lead to early embryonal death, abortion, and sporadically to pyometra resulting in some cases in permanent infertility (Honigberg 1978; Yule et al. 1989). The parasite was first observed by Künstler (1888) and Mazzanti (1900). However the name *Trichomonas foetus* was given to this organism by Riedmüller (1928). Wenrich and Emmerson (1933) transferred the species to the newly established genus *Tritrichomonas*, which was later generally accepted in parabasalid taxonomy (BonDurant and Honigberg 1994).

Tritrichomonas suis is a name given to trichomonads from the nasal cavity, stomach, small and large intestine, and cecum of pig. The earliest report of trichomonads in the porcine stomach was by Gruby and Delafond (1843), and the organisms was named *Trichomonas suis* by Davaine (1877). The presence of trichomonads in the nasal cavity of swine has been reported by Switzer (1951). He also suggested an etiologic relationship between the nasal trichomonad and atrophic rhinitis, which stimulated active research on this subject (Brion and Cottereau 1954; Spindler et al. 1953). Further studies, however, did not confirm the causal association of trichomonads with the porcine disease, and other etiologies of atrophic rhinitis have been identified (Backström 1992; Runnels 1982). Porcine trichomonads are now considered to be harmless commensals (BonDurant and Honigberg 1994).

In spite of the fact that *T. foetus* and *T. suis* were originally described as separate species inhabiting specific sites in two different hosts, controversies about their taxonomic relationship have persisted for almost 50 years. Detailed morphological studies of *T. suis* (Buttrey 1956; Hibler et al. 1960; Switzer 1951) and *T. foetus* (Kirby 1951; Wenrich and Emmerson 1933) revealed a close similarity between these two organisms. More importantly, cross-infection of cows and pigs with heterologous trichomonads excluded strict host specificity of the parasites (Fitzgerald et al. 1958a; Fitzgerald et al. 1958b; Hammond and Leidl 1957; Kerr 1958). The similarities between cattle and porcine trichomonads were further observed in their physiological properties (Doran 1957, 1959; Lindblom 1961; Mattos et al. 1997; Pakandl and Grubhoffer 1994) and antigenic characteristics (De Carli and Guerrero 1975, 1976, 1977; Robertson 1960; Sanborn 1955). More recently, the identity of the two species was suggested by Felleisen (Felleisen 1998; Felleisen et al. 1998) and Vaňáčová et al. (1997) employing techniques of genomic analysis. These reports strongly suggested that the separation of cattle and porcine trichomonads as distinct species is not justified. Nevertheless, unequivocal revision of their taxonomic status has not yet been done.

In this paper we (1) present additional data based on morphological studies, a pathogenicity assay, and DNA analysis of various trichomonad species, demonstrating species identity of *T. foetus* and *T. suis*, (2) summarize and discuss available data concerning identity or differences between the bovine and the porcine trichomonads, and (3) propose a nomenclatural solution for the species name.

MATERIALS AND METHODS

Organisms and cultivation. Origins of the trichomonad strains used for genomic analysis are summarized in Table 1. All organisms are deposited in the cryobank of the culture collection of the Department of Parasitology, Charles University, Prague. Active cultures of the strains were maintained axenically in Diamond's TYM medium (Diamond 1957) supplemented with 10% heat-inactivated horse serum. The pH of the media was adjusted to 6.2 for *Trichomonas vaginalis* or to 7.2 for all other organisms. The trichomonads were cultivated at 37 °C,

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Table **1**. List of trichomonad strains used in this study.

Species	Strain	Host localization	Origin	Isolation
Tritrichomonas foetus	B93	*Bos taurus* prepucium	Bayamo, Cuba	Kulda, 1966
Tritrichomonas foetus	B130	*Bos taurus* prepucium	Bayamo, Cuba	Kulda, 1966
Tritrichomonas foetus	CB-5 clon CB	*Bos taurus* prepucium	Ithaca, NY, USA	Kulda, 1967
Tritrichomonas foetus	CO-1 clon CO	*Bos taurus* prepucium	CO, USA	McLaughlin, 1967
Tritrichomonas foetus	DK-2 (ATCC 30231)	*Bos taurus* prepucium	Davis, CA, USA	Kenrick, 1962
Tritrichomonas foetus	KV-1 (ATCC 30924)	*Bos taurus* prepucium	Žalmanov, CZ	Lípová, Kulda 1957
Tritrichomonas foetus	KV-cl clon KV-1	*Bos taurus* prepucium	Žalmanov, CZ	Lípová, Kulda 1957
Tritrichomonas foetus	LIL-1 clon LIL	*Bos taurus* prepucium	Lublin, Poland	Stepkowski 1970
Tritrichomonas foetus	LUB	*Bos taurus* rectum	Lublin, Poland	Stepkowski 1965
Tritrichomonas foetus	UT (ATCC 30232)	*Bos taurus* prepucium	UT, USA	McLaughlin, 1967
Tritrichomonas foetus	UT-1 (ATCC 30233)	*Bos taurus* preputium	UT, USA	McLaughlin, 1967
Tritrichomonas suis	C19F (ATCC 30169)	*Sus scrofa* cecum	Logan, UT, USA	Hibler, 1959
Tritrichomonas suis	PC-6	*Sus scrofa* cecum	Prague, CZ	Kulda, 1964
Tritrichomonas suis	PC-8	*Sus scrofa* cecum	Prague, CZ	Kulda, 1964
Tritrichomonas suis	PC-9	*Sus scrofa* cecum	Prague, CZ	Kulda, 1964
Tritrichomonas suis	RND	*Sus scrofa* nasal cavity	Doksany, CZ	Kadlec, 1974
Tritrichomonas suis	SUI-H3b	*Sus scrofa* cecum	Halle, Germany	Tachezy, 1988
Tritrichomonas suis	11/S (ATCC 30168)	*Sus scrofa* stomach	Ames, IA, USA	Buttrey, 1956
Tritrichomonas suis	1/N (ATCC 30167)	*Sus scrofa* nasal cavity	Ames, IA, USA	Buttrey, 1956
Tritrichomonas augusta	LV2	*Lacerta vivipara* cloaca	Olešník, CZ	Kulda 1992
Tritrichomonas mobilenis	M776 (ATCC 501616)	*Saimiri boliviensis boliviensis* rectum	Bolivia	Pindak 1984, obtained from Culberson 1989
Tritrichomonas nonconforma	R114	*Anolis bartchi* cloaca	San Vincente, Cuba	Kulda, 1966
Trichomonas vaginalis	C1:NIH (ATCC 30001)	*Homo sapiens* vagina	USA	Jacobs, 1956
Trichomonas vaginalis	TV10-02	*Homo sapiens* vagina	Prague, CZ	Kulda 1973
Trichomitus batrachorum	BUB	*Bufo bufo* cloaca	Veselí nad Lužnicí, CZ	Kulda 1983

except those isolated from lizards and amphibians, which were maintained at 26 °C.

Light microscopy. Moist films spread on coverslips were prepared from soft pellets of trichomonads obtained by centrifugation of cultures in TYM medium without agar. The films were fixed in Hollande's fluid and stained with protargol (Proteinate d'argent, Roques, St-Ouen, France) according to the protocol recommended by Nie (1950). Alternatively, films were fixed in Schaudinn's fluid and stained with Heidenhein's iron haematoxylin or Gomori Trichrome Stain. Trichomonads were observed in a Olympus BX51 microscope using immersion objective 100× UP/PlanApo. Measurements were made with a calibrated ocular micrometer. Interphase cells only were selected for measurement. Cells displaying morphological signs of cytokinesis, such as mastigont duplication and assembly of extranuclear spindle, were excluded.

Transmission electron microscopy. A suspension of trichomonads from culture was fixed in 2.5% (v/v) glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2) on ice for 35 min. After washing in 0.1 M Na-cacodylate buffer the cells were postfixed in 2% (w/v) osmium tetroxide in the same buffer for 1 h. Fixed samples were dehydrated in an ethanol series, transferred to propylene oxide, and embedded in Epon /Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Phillips EM 420 electron microscope.

Virulence assays. The pathogenic potential of the pig and cattle trichomonads was compared using virulence assays on mice. The subcutaneous mouse assay (Honigberg 1961) based on measurement of 6-day abscesses produced by subcutaneous inoculation of trichomonads was performed as described in Kulda (1990). Inbred C57BL mice (males 18–20 g) were inoculated in both flanks with 8×10^5 trichomonads suspended in 0.5 ml of thioglycolate fluid medium. On day 6 after inoculation, the length, width, and height of the lesion was measured and the vol. of the lesion, corresponding to one-half of a spheroid was calculated. Calculations of the mean vol. of lesions produced by individual strains were based on measurements of at least 30 intact lesions.

DNA isolation. A modified guanidium thiocyanate method was used for isolation of DNA (Bowtell 1987). Briefly, trichomonads were mixed with 2 vol. of lysis buffer containing 6 M guanidium thiocyanate, 0.5% (w/v) lauryl-sarcosinate, 10 mM EDTA, 100 mM Tris-HCl, pH 8. DNA was precipitated with 0.4–0.5 vol. of isopropanol, dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8), treated with proteinase-K (50 μg/ml), phenol-extracted, treated with RNAase (100 μg/ml), phenol-extracted, and recovered by ethanol precipitation.

Restriction fragment length polymorphism analysis (RFLP). About 20 μg of the DNA was digested with 10 U of restriction endonuclease (i.e. Apa I, Bam HI, Bgl II, Eco RI, Bsp I) in 25 μl of reaction mixture at 37 °C for 3 h. The restriction digestion was terminated by adding 7 μl of loading buffer. The digestion products were electrophoretically separated in a 1.2% (w/v) agarose gel and stained with ethidium bromide.

PCR-based analysis of variable-length DNA repeats. Variable-length DNA repeats were amplified using the TR7 (5′-CTG TTG TCG ACG TTT ATC CA-3′) and TR8 (5′-GAT CAC CAG TGG AGG GTG TC-3′) primers designed by Riley et al. (1991) and using their PCR procedure. The reaction products were separated by electrophoresis in 3% (w/v) agarose gel and stained with ethidium bromide to observe species-specific fingerprints. Fragments of 502 bp amplified from *T. foetus*, *T. suis*, and *T. mobilensis* genomic DNA were subcloned and sequenced in the forward and reverse direction. The sequences were submitted to GenBank (National Center for Biotechnology Information, NCBI) under Accession numbers AF424696, AF424695 and AF424694.

Randomly amplified polymorphic DNA analysis (RAPD). DNA fragments were amplified by PCR using 29 random prim-

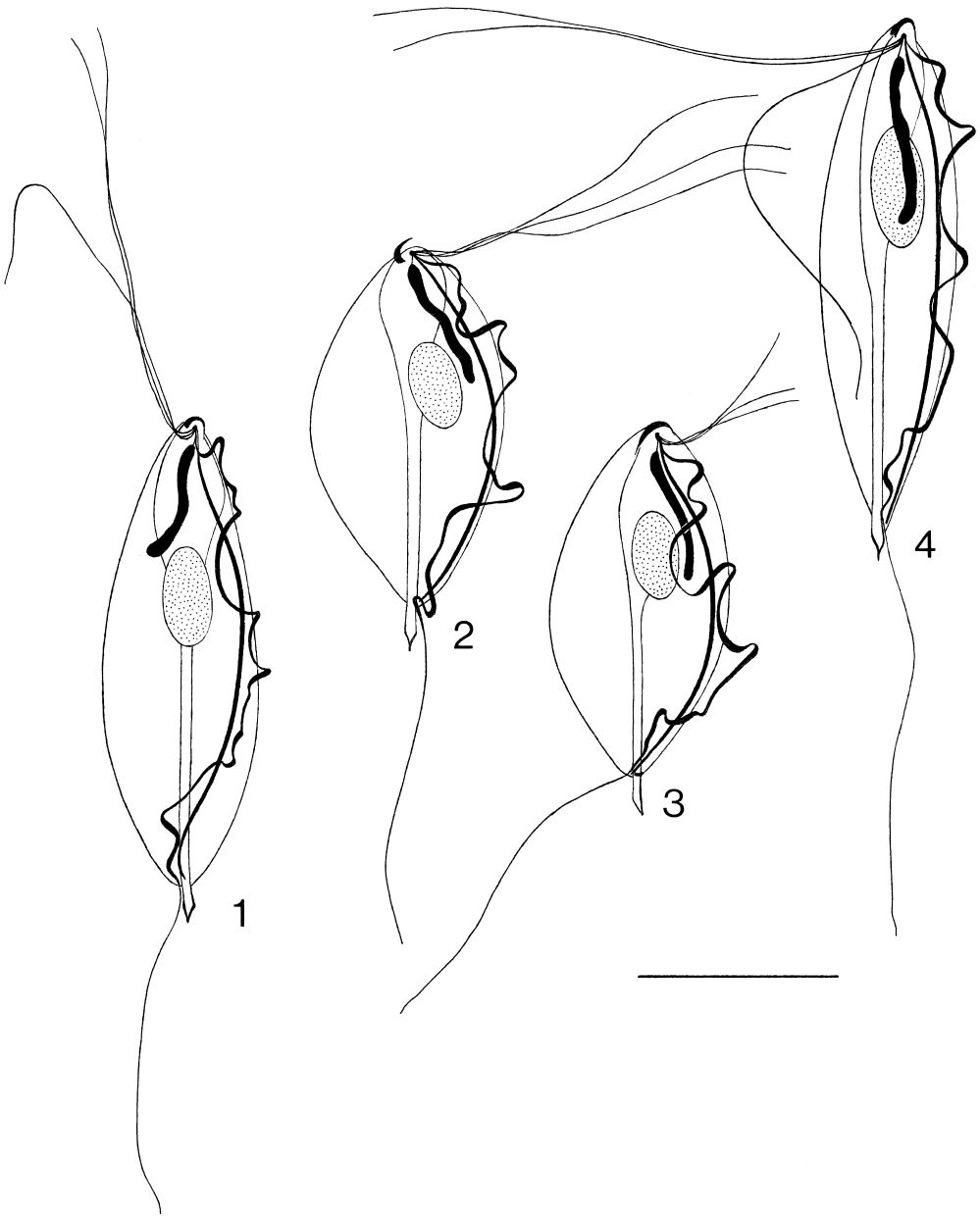

Fig. **1–4**. Representative morphology of *Tritrichomonas foetus* strain LIL (1) and KV-1 (2), and *Tritrichomonas suis* strains PC-9 (3) and PC-6 (4). These composite diagrams of the right lateral view are based on protargol-stained specimens. Anterior flagella on Fig. 3 are not shown in a full length. Bar = 5 μm.

ers (i.e. A01–03, A05, A07–17, A19–20, D16, D19, F01, F03–06, F09–10, F12, F14, and F16) from Operon Technologies Inc., (The University of British Columbia, Vancouver, Canada). The thermocycling was performed in 0.2-ml microtubes containing 10 ng DNA, 5 pmol primer, 2.5 mM $MgCl_2$, 200 μM of each of dNTP, PCR reaction buffer for *Taq* polymerase, and 1 U *Taq* polymerase in total vol. 20 μl. The thermal cycles consisted of 1 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 38 °C, and 2 min at 72 °C. The cycling was terminated after a final 10-min extension cycle at 72 °C. PCR products were electrophoretically separated in a 2% (w/v) agarose gel.

Evaluation of data derived from RFLP and RAPD analysis. The electropherograms were stained with ethidium bromide and photographed with a digital camera ST-7 (Santa Barbara Instrument Group Inc., Santa Barbara, CA). The presence or absence of particular bands was scored with the help of SkyPro CCD Astronomy Software (Software Bisque, Golden, CO) using the pseudocolor rotation function. Genetic distances between DNA samples were computed from Nei's coefficients of similarity $S = 2 \times Mxy / (Mx + My)$, where Mxy, Mx, and My are the number of common fragments between species x and y (Mxy), the number of all fragments (common and unique) of species x (Mx) and of species y (My). The program FreeTree (Hampl et al. 2001) was used for construction of dendrograms by the neighbor-joining (NJ) method and computation of bootstrap values for particular branches of the tree based on 400 replicates.

Analysis of small subunit ribosomal RNA (16S rRNA) genes. DNA was amplified using primers complementary to

Table **2**. Comparison of the body dimensions of *Tritrichomonas foetus* and *Tritrichomonas suis*.

Organism	Length [μm (S.E.)][a,b]	Width [μm (S.E.)][a,b]	Length/ width ratio (S.E.)[a,b]
Tritrichomonas foetus			
KVc-1	11.83 (0.18)	4.32 (0.10)	2.78 (0.07)
LIL	14.72 (0.18)	3.97 (0.10)	3.76 (0.09)
Tritrichomonas suis			
RND	15.00 (0.20)	4.00 (0.10)	3.81 (0.08)
PC-6	14.73 (0.22)	3.43 (0.11)	4.38 (0.11)
PC-9	12.03 (0.14)	4.62 (0.10)	2.64 (0.05)

[a] Mean of 30 cells in μm; S.E., standard error.

[b] Absence of statistically significant differences between *T. foetus* and *T. suis* (length: $F_{(1,3)} = 0.158$, $p = 0.72$; width: $F_{(1,3)} = 0.07$, $p = 0.80$; length/width ratio: $F_{(1,3)} = 0.189$, $p = 0.69$) was confirmed by nested ANOVA with species as independent variable, length, width or length/ width ratio as dependent variables, and strain as nested random factor.

conserved regions near the 3′-end (5′-TACTTGGTTGATCCT GCC) and 5′-end (5′-TGATCCTTCTGCAGGTTCACC) of the 16S rRNA gene (Medlin et al. 1988). The PCR products of 1,569 bp were isolated from an agarose gel using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and subcloned by means of the TA Cloning Kit (Invitrogen, Carlsbad, CA, USA). All genes were sequences in the forward and reverse directions by primer walking. The sequences of *Tritrichomonas foetus* UT, *Tritrichomonas suis* PC-9, *Tritrichomonas augusta* LV2, *Tritrichomonas nonconforma* R114, and *Tritrichomonas mobilensis* M776 were submitted to GenBank (NCBI) under Accession numbers AY055799, AY055800, AY055802, AY055803, and AY055801, respectively. Sequences were aligned using ClustalX (Thompson et al. 2000) and the alignment edited using MUST (Philippe 1993).

RESULTS AND DISCUSSION

Morphology and cell structure. No consistent morphological differences were found between examined strains of *T. foetus* and *T. suis* by us as well as other authors using light (Buttrey 1956; Hibler et al. 1960; Jensen and Hammond 1964) and electron microscopy (Mattos et al. 1997). Both organisms have a spindle-shaped body showing a typical tritrichomonad morphology: three anterior flagella, an undulating membrane reaching the posterior end of the body, a recurrent flagellum continuing beyond the undulating membrane by a free-trailing portion, a small pelta, and an elongate single-arm parabasal (Fig. 1–4). There is some variability in size and shape between the strains, some being slender and more elongate (length/width ratio: 3.8–4.4), and others shorter and wider (length/width ratio: 2.6–2.8). Both types occurred among the examined strains regardless of their pig or cattle origin. In our material the slender type was represented by strains PC-6 (Fig. 4) and RND of *T. suis* and LIL (Fig. 1) of *T. foetus*, the stumpy type by strains PC-9 (Fig. 3) of *T. suis* and KV-1 (Fig. 2) and LUB of *T. foetus*. Measurements of bovine and porcine strains examined by us are summarized in Table 2. Our results fall within the range of measurements obtained by others (Hibler et al. 1960; Randall and Buttrey 1961). A wide range of some published data, especially those regarding cell width (Buttrey 1956; Randall and Buttrey 1961), probably resulted from inclusion of non-interphase cells into the measured sample.

Ultrastructure. The ultrastructure of *T. foetus* and *T. suis* was compared by Mattos et al. (1997) who did not find any differences between the bovine and pig isolates. It has been well established that the ultrastructure of the family Trichomonadidae is genus-specific (Brugerolle 1987) and is of little help in species differentiation. All members of the genus *Tritrichomonas* possess the A-type costa (Fig. 5) and their mastigont contains an infrakinetosomal body, suprakinetosomal body, and the comb (Fig. 7, 8) (Kulda et al. 1987). Only the fine structure of the undulating membrane can serve in differentiating the *T. muris*-type and the *T. augusta*-type groups of tritrichomonads (Brugerolle 1987). Both *T. foetus* and *T. suis* possess the *T. augusta*-type undulating membrane (Fig. 6).

Pathogenic potential and cross-transmission experiments. Mice were subjected to the virulence assay using five strains of *T. foetus* and 3 strains of *T. suis*. All bovine as well as porcine strains established infection after subcutaneous inoculation and induced development of measurable abscesses. Although the mean vol. of lesions caused by individual strains varied over a broad range (29.6–172 mm^3), the size of the lesion did not discriminate tritrichomonads of bovine or porcine origin. In the examined group of strains, the most virulent was the pig strain PC-9 (Table 3). These results show that tritrichomonads from pigs possess pathogenic potential.

Our observations are consistent with earlier studies on trichomonad infectivity and pathogenity for heterologous hosts. Several authors reported successful cross-infection of cattle and swine with *T. foetus* and *T. suis*. Switzer (1951) successfully established vaginal infection in cattle by intravaginal inoculation of cultured *T. suis*. Similar results were reported by Fitzgerald et al. (1958b) and Kerr (1958). Importantly, they observed clinical manifestations of the bovine trichomonosis in experimentally infected animals, and they found that infection of the genital tract of heifers with swine trichomonads is frequently accompanied by infertility. Fitzgerald et al. (1958b) demonstrated transmission of an experimental infection with *T. suis* by coitus, from a heifer to a bull and from the bull to a heifer. Hammond and Leidl (1956) reported infection of the preputial cavity of bulls following inoculation of swine trichomonads and transmission of such infection by coitus. In contrast, Cobo, Cano, and Campero (2001) reported unsuccessful infection of nine heifers with *T. suis* C19F strain. However, interpretation of this latter result is difficult since a control group of heifers inoculated with *T. foetus* was not included in this study. Fitzgerald et al. (1958a) established an experimental infection of pigs with *T. foetus* by inoculating trichomonads isolated from cattle into pig nasal cavities. After 12–24 d the pigs were necropsied, trichomonads were found mainly in the cecum, and occasionally in the small intestine and colon of the pigs.

DNA analysis. Three different methods of DNA fingerprinting and DNA sequencing were used to compare five species of the genus *Tritrichomonas* (*T. foetus*, *T. suis*, *T. mobilensis*, *T. augusta*, and *T. nonconforma*). In some experiments, related species of trichomonads and other parabasalids were added to increase the diversity of the analyzed group and to make possible a comparison of genetic distances among *T. foetus*/*suis* strains with genetic distances among different species of trichomonads.

Restriction fragment length polymorphism analysis (RFLP). This method provides species-specific fingerprints based on the presence of ubiquitous variable-length repetitive sequences in genomic DNA. Characteristic patterns of restriction fragments were observed after digestion of genomic DNA isolated from *T. augusta*, *T. nonconforma*, and *T. mobilensis* (Fig. 9). Common fragment patterns, but different from those of other species, were observed in all strains of *T. foetus* and *T. suis*. Some strain-specific bands were also recognized in *T. foetus* and *T.*

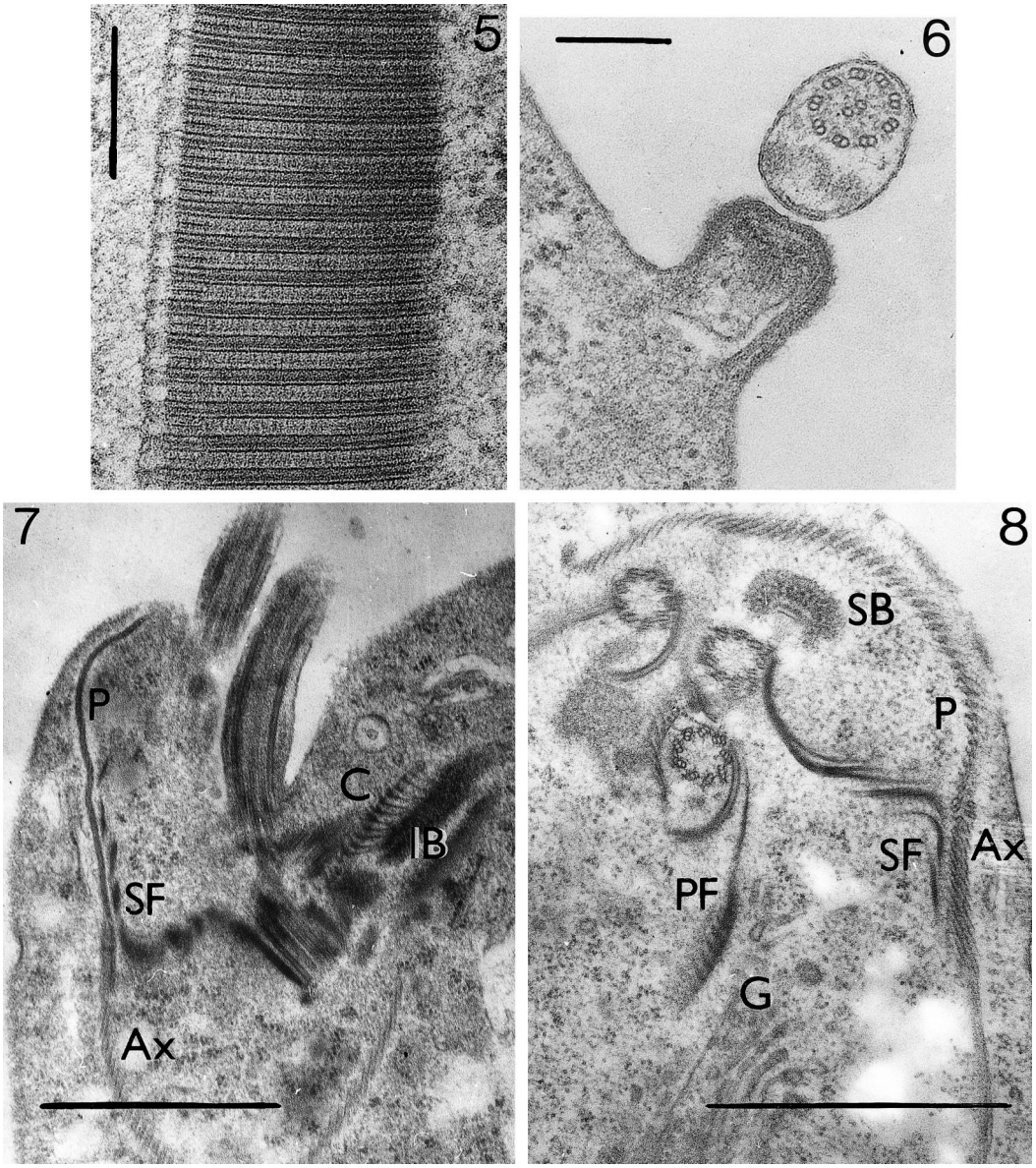

Fig. **5–8**. Typical ultrastructural features common to *Tritrichomonas foetus* and *Tritrichomonas suis*. **5.** Costa of the A-type. A longitudinal section showing the characteristic banding patthern. **6.** Transversal section of the *T. augusta*- type undulating membrane. Bars = 0.25 μm. **7, 8.** Organization of mastigont. Ax: microtubules of the axostyle. C: comb. G: Golgi of the parabasal apparatus. IB: infrakinetosomal body. SB: suprakinetosomal body. SF: sigmoidal fibrils. Bars = 1 μm.

suis. Their presence, however, did not correlate with the bovine or pig origin of the isolates (Fig. 9). Similar results were obtained using 5 restriction enzymes (Apa I, Bam HI, Bgl II, Bsp I, and Eco RI). The similarities between RFLP patterns were used to construct a dendrogram in which *T. foetus* and *T. suis* strains formed a common branch with a bootstrap value of 100% (Fig. 10). Within this branch, the strains of bovine and pig origin were intermixed. *Tritrichomonas mobilensis* appeared as a sister species to the *T. foetus* / *T. suis* branch, while *T. nonconforma* and *T. augusta* formed a more distant group (Fig. 10).

Random amplified polymorphic DNA analysis (RAPD). Similar results were obtained using RAPD analysis, which is based on PCR-amplification of anonymous DNA fragments using short random primers. Similar patterns of amplified DNA fragments were observed in all *T. foetus* and *T. suis* strains, while species-specific patterns were found for other organisms. The data from RAPD analysis using 29 primers, each providing a complex product of about 8–12 fragments, were processed to construct a dendogram (Fig. 11). The high bootstrap values of some internal branches of the *T. foetus* / *T. suis* subtree suggest that RAPD traits do contain information on the cladogenesis of the analysed strains. The general branching pattern of this dendrogram is similar to that derived from analysis of the RFLP data. Both dendrograms indicate that *T. foetus* and *T. suis* belong to a single monophyletic branch, and that strains of both species are intermixed (Fig. 10, 11).

Felleisen (1998) demonstrated the close relationship between bovine and pig strains by means of RAPD analysis using 20 primers from the Operon 10-mer Kit B. The conditions used for RAPD in his study gave simple patterns of 1–5 fragments. Under these conditions he was unable to distinguish bovine

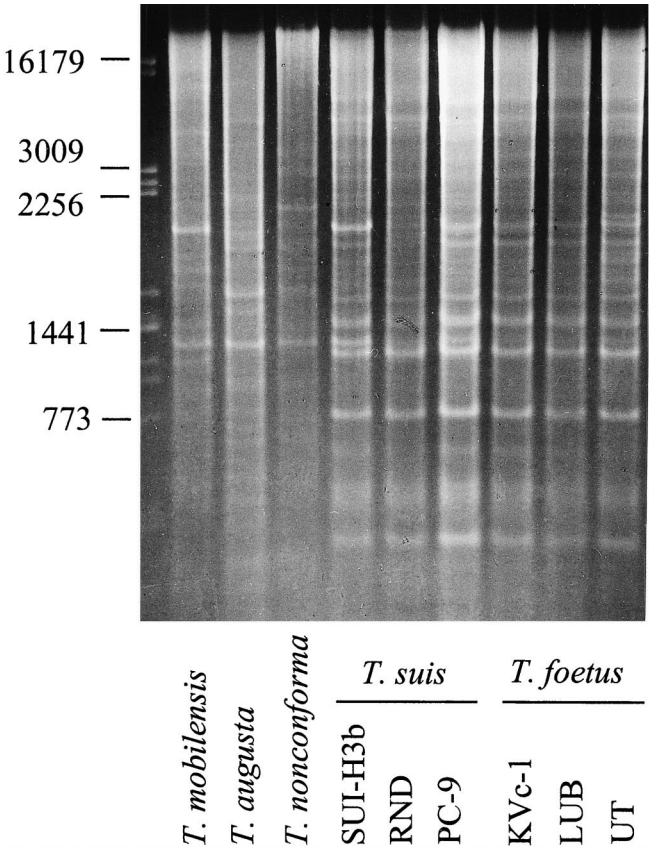

Fig. **9**. RFLP-based analysis of *Tritrichomonas foetus* (KVc-1, LUB, UT), *Tritrichomonas suis* (SUI-H3b, RND, PC-9), and three tritrichomonad species. Genomic DNAs isolated from tritrichomonads were digested by BglII and the restriction fragments were electrophoretically separated in 1.2% (w/v) agarose.

Table **3**. Comparison of the virulence of *Tritrichomonas foetus* and *Tritrichomonas suis* using the subcutaneous mouse assay. Volumes of the lesions were determined 6 days after inoculation of 8×10^5 trichomonads into both flanks of male C57BL mice.

Strain	n[a]	Mean Lesion (mm^2)	Standard Error	Confidence Intervals 95% (mm^2)
T. foetus UT-1	33	29.6	2.24	25.2–34.1
T. foetus CO-1	31	38.0	2.17	33.8–42.3
T. foetus CB-5	34	77.56	3.15	71.4–83.6
T. suis PC-6	30	101.0	3.21	88.0–113.0
T. foetus DK-2	40	106.0	2.78	100.6–111.5
T. suis PC-9	30	126.0	3.21	111.0–144.0
T. foetus KV-1	45	161.9	5.61	150.9–172.9
T. suis PC-8	30	172.0	3.18	115.0–192.0

[a] Number of lesions used for calculation of the mean volume.

strains from those isolated from pig, while *T. mobilensis* displayed different pattern with most of the primers (Felleisen 1998).

PCR-based analysis of variable-length DNA repeats. Under high stringency, the primers designed by Riley et al. (1991) amplified part of the variable DNA repeat elements, which are present in the genome of many unicellular eukaryotic microorganisms (Riley et al. 1991, 1995). Common patterns were observed in all *T. foetus* and *T. suis* strains, which were different from those of other species (Fig. 12). The species closest to *T. foetus*—*T. suis* fingerprint was *T. mobilensis* presenting identical prominent bands at 110, 210, 320, and 502 bp. Due to this remarkable similarity, we further compared the nucleotide sequences of 502-bp fragments amplified from these organisms: bovine and pig strains had identical sequences of 502 nucleotides, while *T. mobilensis* showed 5 different nucleotides (alignment is available from the authors upon request).

Analysis of ribosomal RNA genes. Alignment of 16S rRNA gene sequences (1,530 bp) revealed 100% identity between *T. foetus* UT and *T. suis* PC-9 strains. However, *T. mobilensis* also displayed an identical sequence, and a high degree of the sequence identity was found for *T. augusta* (98.3%), and *T. non-*

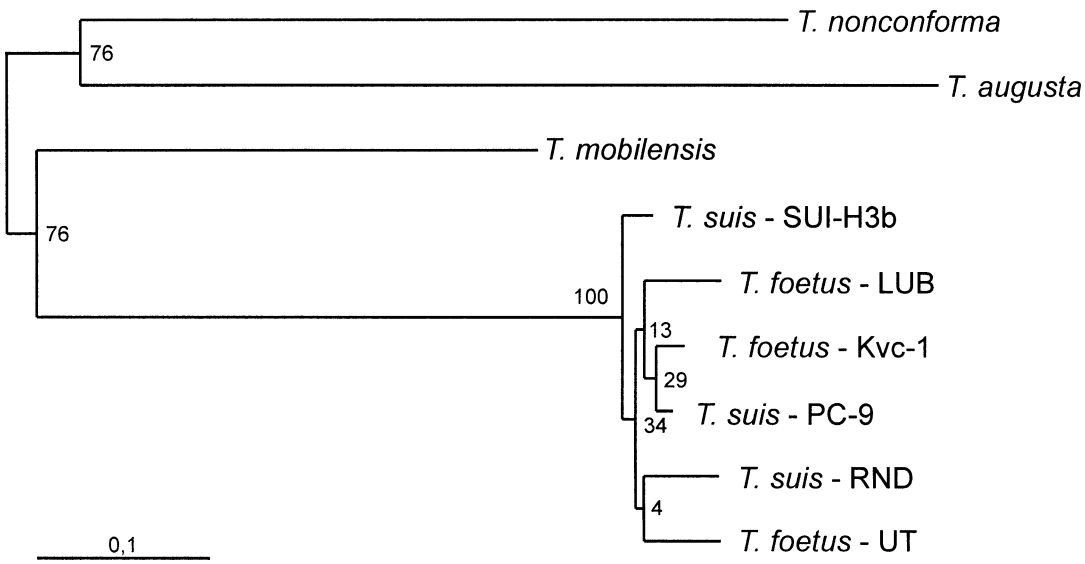

Fig. **10**. Dendrogram constructed on the basis of RFLP analysis using five restriction enzymes, Apa I, Bam HI, Bgl II, Eco RI, and Bsp I. The FreeTree program was used to construct the dendrogram using neighbor-joining method. Numbers indicate bootstrap values for the particular branches of the tree based on 400 replicates.

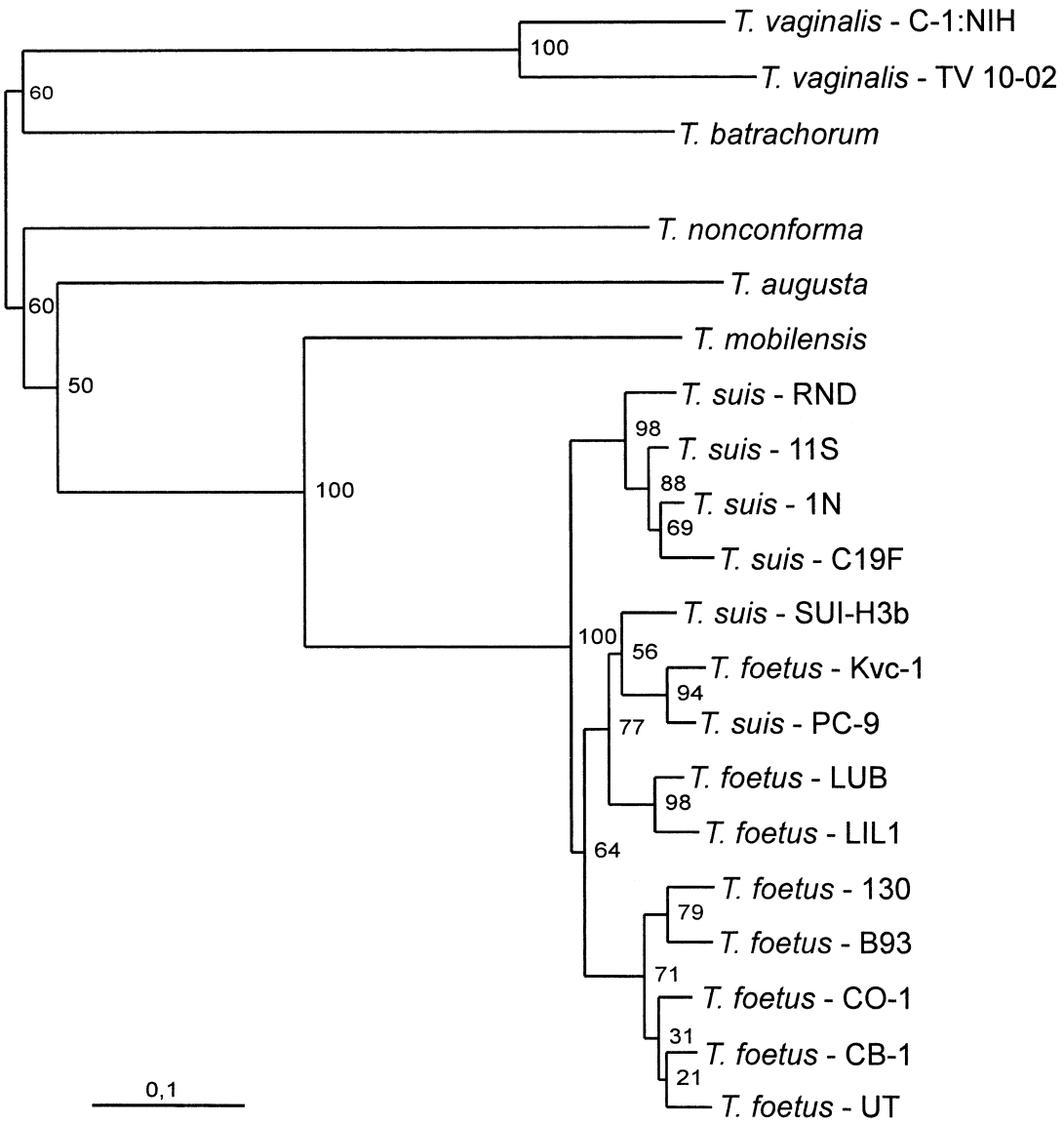

Fig. **11**. Dendrogram based on a RAPD analysis of eight *Tritrichomonas foetus* strains, six *Tritrichomonas suis* strains, and five other trichomonad species. The data from 29 RAPD analyses were used to construct the dendrogram using the FreeTree program. Numbers indicate bootstrap values for the particular branches of the tree based on 400 replicates.

conforma (98.1%) (alignment is available from authors upon request). Similarly, Felleisen (1997, 1998) did not find any differences between eight *T. foetus* and three *T. suis* strains comparing the nucleotide sequences of the 5.8S rRNA genes and the flanking internal transcribed spacer regions ITS1 and ITS2 (372 aligned positions). *Tritrichomonas mobilensis* differed only in one nucleotide from *T. foetus*/*suis*. Although these data further support identity of *T. foetus* and *T. suis*, such high sequence homogeneity among tritrichomonads was not expected. A plausible explanation is that tritrichomonad species diverged so recently, that differences between *T. foetus*/*suis* and *T. mobilensis* do not manifest on the 16S rRNA gene level.

Physiological and immunological properties. In addition to previous analyses, several studies have compared *T. foetus* and trichomonads isolated from pigs in certain aspects of their metabolism. Doran (1957, 1959) and Lindblom (1961) found only slight quantitative and qualitative differences in utilization of metabolic substrates, production of acids, production of hydrogen, respiratory activity, certain enzymatic activities, and effects of metabolic inhibitors. *Tritrichomonas suis* isolated from the nasal cavity was more similar to *T. foetus* in its biochemical properties than were the cecal strains. As suggested by Doran (1959) and Honigberg (1978), the observed differences were compatible more with intraspecific variation than variation between species. More recently, Pakandl and Grubhoffer (1994) showed similarities between bovine and pig trichomonads in their sialic-acid binding system. Mattos et al. (1997) compared 5 species of trichomonads including five *T. foetus* and three *T. suis* strains using isoenzyme analysis. They found only subtle differences between bovine and swine trichomonads, differences no larger than those observed between different strains of *Trichomonas gallinae*.

Common antigens or closely related antigenic structures are found in bovine and pig trichomonads. Robertson (1960) and De Carli and Guerrero (1975, 1976, 1977) demonstrated cross-reactivity of immune rabbit sera between *T. foetus* and *T. suis*

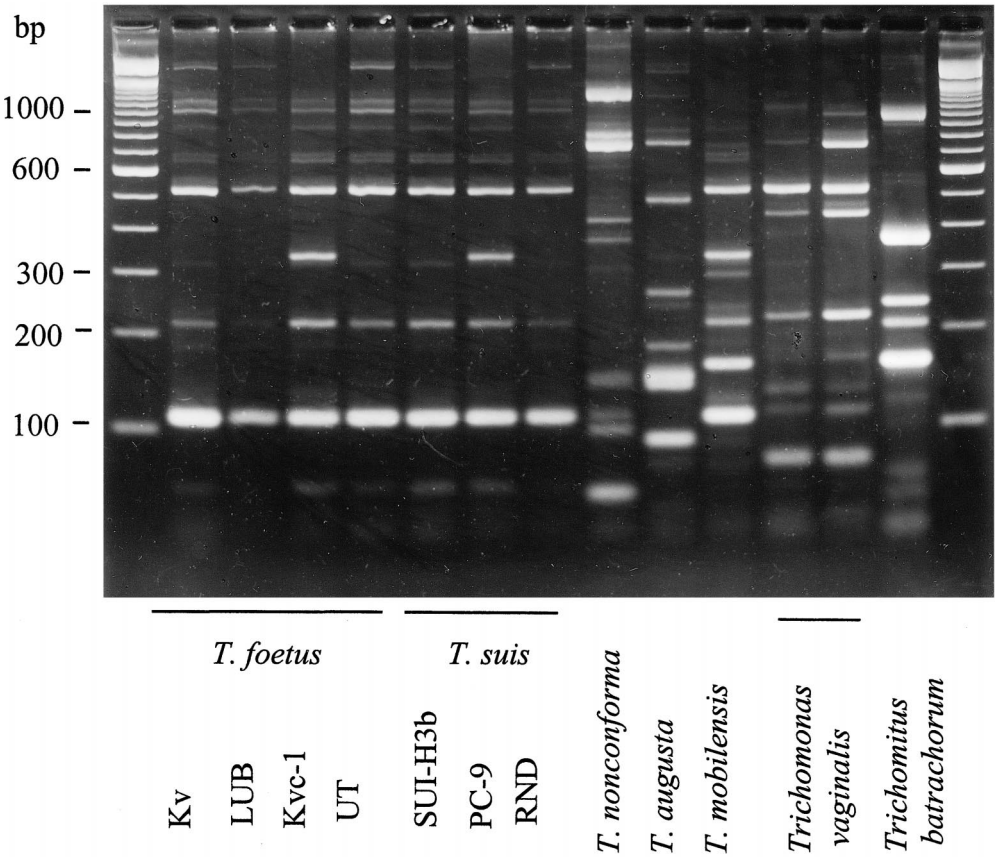

Fig. **12**. PCR analysis of variable-length DNA repeats using TR7/TR8 primers. Common patterns were observed in all *Tritrichomonas foetus* and *Tritrichomonas suis* strains, which were different from those of other species. Strain-specific bands recognized in *T. foetus* and *T. suis* did not correlate with the bovine or pig origin of the strains.

using precipitation and agglutination tests, indirect immunofluorescence, gel immunodiffusion, and immunoelectrophoresis. Kerr (1958) obtained positive results in an agglutination test with both organisms using mucus from heifers intravaginally infected with *T. suis*. However, antigenic differences between bovine and pig trichomonads have been found by Sanborn (1955) with the aid of an agglutination test. Nevertheless, two strains of *T. suis* isolated from the nasal cavity and cecum were as different from each other as each was from *T. foetus*. More recently, Cobo, Cano, and Campero (2001) used an ELISA-assay to show strong cross-reactivity against bovine and porcine tritrichomonad strains of the serum from heifers vaccinated with *T. foetus*.

Under the biological species concept, a species usually is perceived as a reproductive community whose gene pool retains coherency primarily via the bonds of interbreeding and genetic exchange. This concept is not applicable for protists and other unicellular organisms consisting of clonal or semiclonal populations. Such species are usually described on the basis of morphology, host specificity, pathogenicity, other epidemiological and physiological characters, and phylogenetic divergence from related organisms (Tibayrenc 1998). Original descriptions of *T. suis* and *T. foetus* were based on their morphology in light microscopy and their presence in different organs of different hosts. Further studies by various authors as well as by us revealed that the morphology of bovine trichomonads and *T. suis* is identical. Few differences have been found in their biochemical and immunological properties. Both organisms have pathogenic potential, are cross-infective between cattle and pig, and contain strains of similar virulence as determined by a mouse assay. Studies of genomic DNA showed a high degree of genetic similarity between *T. foetus* and *T. suis* strains using three methods of multilocus analysis (i.e. RFLP, RAPD, and PCR-based analysis of variable-length DNA repeats). Phylogenetic analysis of RFLP and RAPD data derived comparable dendrograms indicating that *T. foetus* and *T. suis* belong to a single monophyletic group. Finally, no differences were found in sequences of 16S rRNA genes, 502 nucleotides of DNA fragments, as well as in the ITS1–5.8SrRNA-ITS2 region (this study, Felleisen 1997). All these findings lead to the conclusion previously suggested by other authors (Felleisen 1997; Hibler et al. 1960; Honigberg 1978; Mattos et al. 1997) that *T. foetus* and *T. suis* belong to the same species.

Recognition of species identity of the bovine and pig tritrichomonads has important epizoonotic implications. Bovine trichomonosis is considered an eradicated infection in many European countries where controlled insemination has been introduced. However, the cattle pathogen is present in the intestines of pigs, hidden under the name *T. suis*. Natural infection of pigs is rather frequent. In a report from the Czech Republic (Pakandl 1994), 68–90% prevalence was found in various groups of 149 porcine heifers and sows and 77% prevalence in 142 examined gilts. The existence of a ''natural'' reservoir and demonstration of infectivity of the porcine tritrichomonads for cattle (Fitzgerald et al. 1958b) points to a risk of reintroduction of trichomonosis to bovines, when pigs and cattle are maintained together. Different species names that are in use for the

pig and cattle parasites pose an obstacle for comprehending such a possibility.

According to the principle of priority (Article 23 of the International Code of Zoological Nomenclature), the name *T. suis* (Gruby and Delafond 1843) has priority over *T. foetus* (Riedmüller 1928), but renaming of the cattle trichomonad would cause considerable confusion in the veterinary and parasitological literature. To avoid this, we propose with reference to the Article 23.9.3 of the Code (Ride et al. 1999), to suppress the older name *suis* and maintain its junior synonym *foetus* as a *nomen protectum* for both cattle and porcine trichomonads. This proposal is supported by the fact that the vast majority of published experimental work, as well as the cattle disease and its veterinary and economic significance, is associated with the name *T. foetus*. The case has been referred to the International Commission on Zoological Nomenclature for ruling under its plenary power.

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