On the identification of some *Entamoeba* species. Comments on a recent paper

In our opinion, the recent article by Clark *et al.* (2006) on the phylogeny of *Entamoeba* species by genetic analysis contributes more confusion than clear insights to the systematics of this genus, especially in relation to *Entamoeba equi* and *Entamoeba struthionis*.

Trophozoites and four-nucleated cysts of *E. equi* were described by Fantham (1921) from two horses with intestinal disturbance. No cysts were found in the horse stool sample analysed by Clark *et al.* (2006) and there is no description of the trophozoites found in culture [the paper they refer to (Clark & Diamond, 2002) is only a revision of culture methods]. It is impossible to confirm whether or not these trophozoites are compatible with Fantham's description or whether they really form four-nucleated cysts. Furthermore, despite environmental contamination being detected in the sample (*Colpodella* spp. were also found), the authors did not explain why this possibility was not taken into consideration for the *Entamoeba* trophozoites (especially when no cysts were found in the sample). In our opinion, the only valid identification that applies is *Entamoeba* sp., and the genetic characterization and validation of *E. equi* should wait until new isolates are obtained.

In relation to *E. struthionis*, its differential morphological, biological (Martínez-Díaz *et al.*, 2000) and genetic (Ponce-Gordo *et al.*, 2004) characteristics have been ignored by Clark *et al.* (2006) on the basis that a partial sequence of the small-subunit rRNA gene of a pig isolate 'showed close similarity to *E. polecki*' and it is one of those genetic variants of *Entamoeba polecki* the existence of which 'has become clear'. However, there is no such close similarity, and *E. polecki*

genotype variants have not been described. There are 36 differences (including base substitutions and deletions) in the partial sequence between *E. struthionis* and *E. polecki* (39 between the pig isolate and *E. polecki*), all of them located in the variable (taxonomic) regions of the gene. A comparable degree of dissimilarity is found in other *Entamoeba* species comparisons, such as *Entamoeba chattoni*–*E. polecki* (45 differences along the same fragment), *E. chattoni*–*E. struthionis* (48 differences) or *Entamoeba histolytica*–*Entamoeba dispar* (22 differences). With respect to the existence of genetic variants in *E. polecki*, this could also apply to *E. struthionis*, where not only genetic but also morphological differences are documented. Anyway, no genetic variants in *E. polecki* have been described to date. The references the authors give to demonstrate the existence of such variants are incorrectly used; Verweij *et al.* (2001) described two unidentified genotypes of one-nucleated cyst-forming *Entamoeba* from human samples as *E. polecki*-like (but not as *E. polecki* genotypes), and Blessmann *et al.* (2002) carried out an epidemiological study in which *E. polecki* (or other one-nucleated cyst-forming *Entamoeba* species) was not considered at all. In our opinion, the only relevant information from the article of Clark *et al.* (2006) in relation to *E. struthionis* is the detection of the species in a pig; however, as this observation was made in one sample from one pig, a spurious parasitosis cannot be ruled out on the basis of the data available.

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