

Genetic and Phylogenetic analysis demonstrate wild Ethiopian ostriches infection with
Libyostrongylus dentatus (Nematoda, Trichostrongylidae)

Josiana Gomes de Andrade¹; Bersissa Kumsa^{2,3}; Dinka Ayana⁴; Clóvis de Paula Santos¹; Alena Mayo Iñiguez⁵; Renato Augusto DaMatta¹

¹Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Rio de Janeiro, Brazil;

²Department of Parasitology and Pathology, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia; ³Aix Marseille Université, Marseille, France; ⁴Dean College of Veterinary Medicine & Agriculture, Addis Ababa University, Bishoftu, Ethiopia; ⁵LABTRIP, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz-Fiocruz, Rio de Janeiro, Brazil.

Ostriches (*Struthio camelus*) are native birds from Africa and different subspecies are distributed in the continent. In Ethiopia, it is found the wild species *Struthio camelus molibdophanes*. While in the American continent, including Brazil, the domestic species, *Struthio camelus domesticus* is mainly found. Ostriches are infected with different nematode species, however *Libyostrongylus* spp. is the most pathogenic and can be lethal. In most cases, ostriches of Brazilian breeding are infected with two species of this genus, *L. douglassii* and *L. dentatus*. To understand this mixed infection and to confirm morphometric data, the presence of *Libyostrongylus* spp. in faeces of wild Ethiopian ostriches was investigated genetically. Faecal samples of five ostriches were cultivated to obtain infective larvae (L₃) of *Libyostrongylus* spp. that were sent to Brazil. By optical microscopy L₃ were identified according to morphometric parameters. A pool of 50 specimens from each of 5 hosts was used to extract DNA. The first and second internal transcribed spacer (ITS-1 and ITS-2) of the nuclear ribosomal DNA was amplified, sequenced and analyzed. Genetic distances were calculated using Kimura 2-parameter. ITS-1 and ITS-2 phylogenetic trees were constructed using Maximum Likelihood with Tamura 3-parameter model and Neighbor-Joining with Kimura 2-parameter model. Genetic distance data based on ITS-1 and ITS-2 showed that *Libyostrongylus* sp. from Ethiopia belong to the *L. dentatus* species. The same genetic distance between *Libyostrongylus* sp. from Ethiopia and *L. douglassii* was verified between Brazilian *L. dentatus* and *L. douglassii*. The phylogenetic trees based on ITS-1 and ITS-2 showed that *Libyostrongylus* sp. from Ethiopia clustered with Brazilian *L. dentatus* sequences in a monophyletic clade with high bootstrap values. Molecular data based on ITS-1 and ITS-2 sequences confirmed morphometric data indicating that the *Libyostrongylus* sp. from Ethiopia belongs to the *L. dentatus* species. In addition, the study supports that mixed infection of *Libyostrongylus* species found in domestic ostriches may be related to the interbreeding of wild ostrich subspecies.