

A L S L S L I Y Y D I V S Y V V M L C F F M C V L T V F Y L L F L G F F S G N V I L N K E F N N L E F 50
 B L S L S L I Y Y D I V S Y V V M L C F F M C V L T V F Y L L F L G F F S G N V I L N K E F N N L E F
 C L S L S L I Y Y D I V S Y V V M L C F F M C V L T V F Y L L F L G F F S G N V I L N K E F N N L E F

A K W T F I P S L F M S I L C Y L N L S F F Y F D S E L D T I S I V K V M G R Q W Y W S Y E D S F K G 100
 B K W T F I P S L F M S I L C Y L N L S F F Y F D S E L D T I S I V K V M G R Q W Y W S Y E D S F K G
 C K W T F I P S L F M S I L C Y L N L S F F Y F D S E L D T I S I V K V M G R Q W Y W S Y E D S F K G

A L Y D S Y H L S S L I N S V D N P L V L T Y N S S T R I L M S S S D V L H S F S V P D L G L K M D A 150
 B L Y D S Y H L S S L I N S V D N P L V L T Y N A S T R I L M S S S D V L H S F S V P D L G L K M D A
 C L Y D S Y H L S S L I N S V D N P L V L T Y N S S T R I L M S S S D V L H S F S V P D L G L K M D A

A V P G R V N H L T Y L P T R V G S F M G Y C S E L C G V G H S F M P M S V E V I I 191
 B V P G R V N H L T Y L P T R V G S F M G Y C S E L C G V G H S F M P M S V E V I I
 C V P G R V N H L T Y L P T R V G S F M G Y C S E L C G V G H S F M P M S V E V I I

Fig. S1. Amino acid consensus sequences for the cytochrome oxidase II (COII) gene from four species of *Gyrodactylus* collected from *Scleromystax barbatus* and *Scleromystax macropterus*. (A) The consensus sequence of the COII gene derived from haplotypes of *Gyrodactylus bueni*, *Gyrodactylus major*, *Gyrodactylus scleromystaci*, and *Gyrodactylus* sp. (B) The consensus sequence for COII obtained from haplotypes of *Gyrodactylus scleromystaci* from the sub-basin of the Nhundiaquara River. (C) The consensus sequence for COII derived from haplotypes of *Gyrodactylus scleromystaci* from the sub-basin of the Paranaguá Bay. Letters within squares correspond to sites evolving under stronger purifying selection (scale 6-7 by Selecton). Letters with grey background represent sites evolving under positive selection (scale 1-2 by Selecton). Remaining letters correspond to sites evolving under weaker purifying selection (scale 3-5 by Selecton).