Horizontal transfer events might be the responsible for the lack of congruence between teleosts and *Rex* phylogeny. Indeed pathogens, parasites, and other vectors could have favored the regional homogenization of *Rex* retroelements in species sharing the same habitat but phylogenetically distant [15,23-26]. Consequently, besides the relationship between temperature and genetic distance of *Rex3* sequences, it is interesting to evaluate the relationship between genetic distance and geographical localization of considered species even if a limitation is due to the wide distribution of some species. To this aim we performed a variance partitioning analysis using vegan package 2.5.5 [14].

Data of genetic distance are referred to sequence pairwise thus, for each pair of specimens, sequence divergence has been compared with the belonging to cold or warm waters and to physical distance (Electronic supplementary material, figure S1, panel A). The belonging to cold or warm waters has been ascribed for each pair of specimens assigning the following characters: 0 if both specimens were of cold waters, 1 if both specimens were of warm waters, and 2 if specimens were of different water temperatures.

Moreover, to include the information of the phylogenetic analysis (Figure 2) we performed also an analysis based on sequence topological position in phylogenetic tree compared with the belonging to cold or warm waters and to physical distance (Electronic supplementary material, figure S1, panel B). Sequence topological position in phylogenetic tree has been attributed for each pair of specimens assigning the following characters: 0 if both sequences were of clade A, 1 if both sequences were of clade B, and 2 if sequences were of different clades.

Variation partitioning attempts to "partition" the explanatory power of different explanatory variables in relation to the same response variable. We named X1 and X2 the two explanatory variables considered (for details see caption of Electronic supplementary material, figure S1) and X the response variable. In particular, we performed variation partitioning analysis (VPA) using redundancy analysis (RDA). The result of the analysis is represented by a Venn diagram (Electronic supplementary material, figure S1) in which the partition of the variation of a response variable X between two sets of explanatory variables (X1 and X2) is showed. The bounding rectangle represents the total variation in X. Each circle represents the portion of variation accounted for by an explanatory variable or a combination of explanatory matrices. The intersection between the two circles represents the amount of variation explained by the two variables X1 and X2 (Legendre et, 2008).

In the panel A value indicating the correspondence between the belonging to cold or warm waters and genetic distance is slightly higher than that indicating the correspondence between physical distance and genetic distance. This finding does not allow to rule out the possibility that horizontal transfer had contributed to incongruence between teleosts and Rex3 phylogeny. However, in the panel B an explicit correspondence between sequence topological position in phylogenetic tree and the belonging to cold or warm waters is evidenced. The testable fractions of each analysis performed have been statistically tested and showed a p<0.001.

Therefore, the results here obtained suggest that the evolutionary pattern of *Rex3* retroelements has been influenced by water temperature and not by horizontal transfer events.

Reference:

Legendre P. 2008 Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis*. J Plant Ecol.* **1**: 3-8.