The evolutionary relationships in Xenopus genus are intensively studied for its interspecific variability and high conservation in evolution. These characteristics possess an opportunity for comparative studying of polyploidization phenomenon on interchromosomal level and an occasion to identify the genome-forming mechanisms with cytogenetic methods. XME chromosomes (X. mellotropicalis, 2n=40) were identified via p-/q- arm length ratio in a comparison with morphometric analysis of X. epitropicalis (2n=40) chromosomes. Whole chromosome painting probes were prepared from X. tropicalis (2n=20) microdissected chromosomes and they were applied to XME metaphase spreads via optimalized Zoo-FISH. 10 chromosomal quartets were detected and one balanced non-reciprocal translocation between chromosomes XME 2 and XME 9 which must have occurred in a diploid ancestor. Thus, we disprove the theory of Silurana subgenus origin via only one polyploidization event.