NGS approach for investigating evolutionary transition from oviparity to viviparity in squamate reptiles.

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Introduction
The lizard Zootoca vivipara (Figure 1) is one of the few example in Nature which shows, within the same species, populations with different reproductive modalities. Oviparous populations live in the southern part of its distribution range (the newly discovered Zv.carniolica in Eastern Italian Alps and Z.v.louisianzzi in the Pyrenees), while viviparous subspecies (e.g. Z.v.vivipara and Z.v.sachalinensis) are widely distributed from British Isles and central France to Scandinavia and north-eastern Asia (Figure 2) [1]. This species is, therefore, particularly well suited for studying the evolutionary shift in reproductive mode.

Results and Discussion
Bioinformatic analysis performed using the pipeline software Stacks produced about 100.000 local alignments and about 260.000 Single Nucleotide Polymorphisms (SNPs). 87.385 SNPs, selected from the 75bp single-end reads showing no more than 5 SNPs, were used to describe the overall genetic variation between subspecies. The result of MDS (Figure 2) seems to confirm [according to [1]] the existence of two oviparous clades (Z.v.carniolica and Z.v.louisianzzi) and one viviparous clade (composed by Zv.vivipara and Z.v.sachalinensis).
We additionally analyzed a restricted marker-set (408 SNP) in order to have at least 50% of present data in each clade, to better investigate the genetic basis of evolution of viviparity dividing our dataset according to reproductive mode. We adopted three different approaches (Figure 5):

(1) Fst Outlier (intersection of markers with pairwise Fst ≥ 0.5 between vivipara (V) and carniolica (O) and vivipara (V) and louisianzzi (O) and Fst ≤ 0.05 within vivipara (V));
(2) Tassel (genotype-phenotype association using Mixed Linear Model) [3];
(3) Gemma (software implementing the Genome-wide Efficient Mixed Model Association algorithm) [4].

We selected 217 SNPs that were identified in at least two methods. Genomic sequences (200-500 bp long, achieved with Illumina Paired-end protocol) physically linked to these markers were then BLASTed against the Anolis Carolinensis genome [5] (the only reptile genome available at the moment; A. carolinensis and Z. vivipara share the most recent common ancestor about 180 mya).

Materials and Methods
A Next Generation Sequencing technique was used to analyse 40 samples of Zootoca vivipara to cover the overall genetic variation of the species. RADtag sequencing (Figure 3) uses Illumina HiSeq technology to simultaneously discover and analyse thousands of SNPs at genome level [6]. Bioinformatic analyses have been conducted using the pipeline software Stacks v.1.02 [7].

Conclusions
Looking for genes and markers showing signals of selection is becoming relatively straightforward with the advent of NGS; RADtag, together with Paired-end sequencing, is a useful method to assemble millions of genome reads into contigs which can be compared to known sequences in existing databases. However, not all genes can be identified, since non-model species may contain unknown genes or the closest reference genome may not be so close.

We analyzed contigs physically linked to 217 SNPs which showed signal of selection and we found sequence similarities in about 60% of them (max E-value 0.5, against A. carolinensis genome). Among the predicted proteins observed, eight genes could be possibly related to reproductive mode according to previous studies that focused on different physiological pathways that differ between oviparous and viviparous reproductive mode [8]. Evolution of viviparity poses a major immunological hurdle for mother and foetus. For instance, cytokines and other proteins involved in immunological response seem to play a similar role in mammals and viviparous squamates. Hormone receptors have an essential part in evolution of viviparity, in fact they may regulate follicular development and oocyte maturation. Also proteins involved in placental development and vascularization are peculiar elements that characterize mammals and viviparous squamates. Examples of these gene categories were identified in this study and listed in Table 1.

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References