First steps of systematically DNA barcoding in Croatia - example of caddisfly fauna (Trichoptera)

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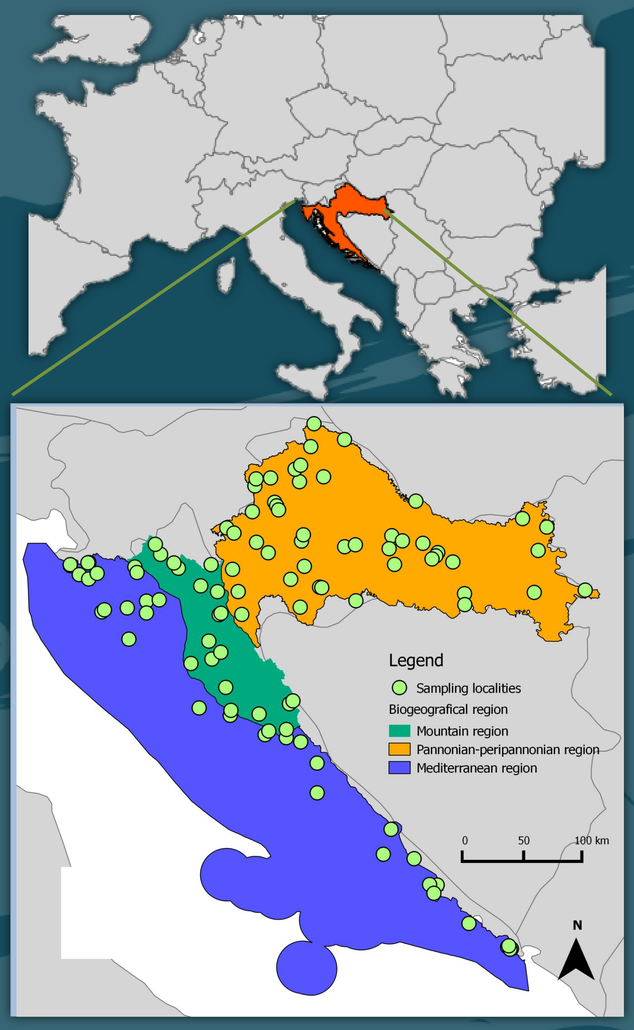
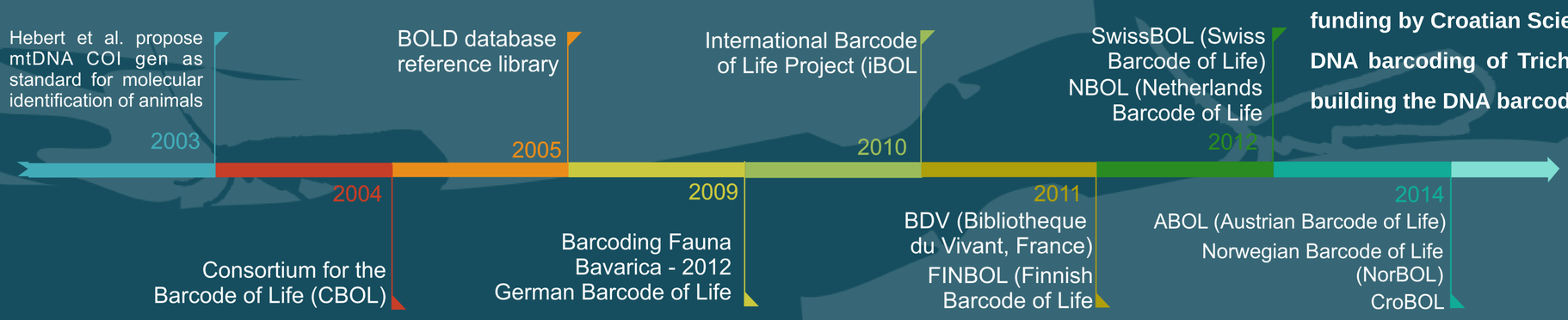
Introduction

Today DNA barcoding is widely used method and most of the research organizations and national government invest in the DNA barcoding initiative towards the ultimate goal of a DNA barcode reference library of all life on Earth. Year after Hebert proposed segment of the mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) gene to be standard barcode region for animals, Consortium for the Barcode of Life (CBOL) was established. There was genetic sequence database - GenBank, but in goal to promote DNA barcoding The Barcode of Life Data Systems (BOLD) was established. Developing DNA barcoding as a global standard for the identification of biological species started the International Barcode of Life Project (iBOL). In several European countries national initiatives are well established while Croatia just starting to DNA barcode of the local biodiversity. Previously, DNA barcode method in Croatia was applied only sporadically without major systematic investigation of any group of animals.



Map of Croatia with sampling location and geographical regions according to Bertić et al 2001 →



Material and methods

Trichoptera inventory was conducted at 125 localities in 3 geographical regions in Croatia since 2014. Samples of adult caddisflies were collected using an entomological net and UV light traps, and larvae were taken by hand. The samples were stored in 96% ethyl alcohol. For determination of collected specimens standard literature was used (Malicky 2004, Waringer & Graf 2011). Genomic DNA was extracted from legs and the remainder of the specimen was kept as a voucher in the Trichoptera DNA Barcode collection in Natural History Museum in Zagreb. DNA was extracted using commercial kits (e.g. Sigma-Aldrichl, Qiagen) according to the manufacturer's specifications. Full-length *COI*-5P DNA barcodes for the most of specimens were amplified using a standard set of primers: LCO1490/HCO2198 (Folmer et al. 1994). In several cases modified Folmer primers (TM1 LCOI, TM2 LCOI, TM3 HCOI, TM4 HCOI) had to be used for successful amplification. PCR cycling conditions were as described in Podnar et al. 2004. Using BOLD Identification Engine all DNA obtained barcode sequences were compared to other caddisfly sequences on BOLD database to locate the closest match. Phylogeny-based identification was conducted by using different methods of tree reconstruction (Neighbor-Joining (NJ), Maximum likelihood (ML) as implemented in MEGA 6.0. and Bayesian Inference (BI) in MrBayes 3.1.2

Results

It is assumed there are around 200 caddisfly species in Croatia, however, hitherto there is no official checklist. We selected list of 188 species according to literature. Up to now, we have DNA barcodes of 127 caddisfly species. There are still 61 species for which DNA barcodes have not been obtained yet. Most of them account to species that we failed to collect. It is case of rare species that have specific habitat preference (e.g. *Hagenella clathrata* inhabiting mainly peatlands) or have small population size (e.g. *Chaetopteryx uherkovichi*). Some species are doubtful for Croatians caddisfly fauna (e.g. *Rhyacophila simulatrix*). In our goal to have Trichoptera DNA barcode reference library, we have succeed to found some first records for Croatia (e.g. rare species: *Adicella cremisa* and *A. balcanica, Tinodes antonioi, Micropterna wageneri*) also revealed the existence of some cryptic species.

Conclusion

In the context of the on-going 6th global biodiversity crisis (mostly referred to as a mass extinction), the major challenge that taxonomists are currently facing is to describe species diversity before it actually becomes extinct. Knowing this and fact that Croatian territory has great habitat diversity and represents one of the European biodiversity hotspots, we have much to loss. In 2017: the project „DNA barcoding of Croatian faunal biodiversity" (CroBarFauna) has been accepted for funding by Croatian Science Foundation. So there is hope that this project DNA barcoding of Trichoptera won't be exemption but just first step in building the DNA barcodes library the complete Croatian biodiversity.