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NEWS AND VIEWS

PERSPECTIVE

Aquatic biodiversity assessment for the lazy

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The world is covered in DNA. In any ecosystem, extracellular DNA fragments can be found that once formed the genomes of a variety of micro- and macroorganisms. A few years ago, it was proposed to use this environmental DNA (eDNA) as a source of information on local vertebrate biodiversity (Ficetola *et al.* 2008; Taberlet *et al.* 2012). This idea offered an elegant solution to take up the gauntlet of rapidly increasing monitoring needs. Coupled with barcoding efforts, it promised to be cost-efficient in many respects, for example man-hours and taxonomic expertise. Ecologists and conservation biologists with an interest in aquatic ecosystems have enthusiastically adopted and pioneered this new method, producing dozens of eDNA studies. Most of these studies have, however, focused on a single or a few aquatic species. In this issue of *Molecular Ecology*, Valentini *et al.* (2016) move the field a step further by demonstrating that metabarcoding approaches – which simultaneously target large groups of organisms such as amphibians or fish – can match and sometimes even outperform other inventory methods.

Keywords: amphibians, biodiversity, environmental DNA, fish, metabarcoding

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Environmental DNA metabarcoding the right way

Any vertebrate eDNA study is an attempt to look for a needle in a metagenomic haystack. One certainly does not want to do that without a clear strategy in mind. Valentini *et al.* (2016) therefore designed their study very carefully. For a start, they built their own high-performance molecular tool box. They selected primer sets based on solid *in silico* predictions and in vitro testing. They then decided to perform sampling and laboratory work under ancient DNA-like (aDNA-like) conditions. It is a very reasonable decision considering that (i) both aDNA and eDNA are potentially degraded and only present in minute amounts in very complex samples and (ii) metabarcoding approaches are based on a very sensitive initial enrichment step, that is a PCR assay, whose product is dissected using very sensitive high-throughput sequencing techniques (Pedersen *et al.* 2015). Sensitivity to contamination is likely to be high, and a serious risk of false detection follows. Being aDNA-like cautious at the bench is thus no luxury with eDNA. Finally, the authors also analysed their sequence data with great care. Environmental DNA sequences are short, which makes their taxonomic assignment a very challenging task. Applying state-of-the-art analytic pipelines is a must (Coissac *et al.* 2012; Boyer *et al.* 2016). In the best case, bioinformatic analyses will be backed on a trustable reference sequence database, and the authors also engaged in a parallel barcoding effort aimed at filling gaps in the published sequence record.

In situ comparison of eDNA metabarcoding and other monitoring techniques

The merits of eDNA can only be appreciated in comparison with other monitoring techniques. In this study, the authors provide a comparison of eDNA metabarcoding and traditional surveys in 'real-world' aquatic ecosystems. Two key vertebrate groups, amphibians and bony fish, were targeted. To keep it short, we will only focus on the results obtained for amphibians; similar trends were observed for bony fish. The authors assessed amphibian diversity at 39 water bodies representing various types of temperate aquatic ecosystems. They sampled water and performed traditional surveys (combining several methods) the same day. They then used their newly designed amphibian minibarcode system to generate eDNA amplicons, which were sequenced on a MiSeq platform. On a per-species basis, eDNA metabarcoding strikingly outperformed traditional surveys. Detection probabilities estimated with occupancy modelling were markedly higher for 10 of 12 species, with values comprised between 0.89–1.00 vs. 0.20–0.71 for traditional surveys. For the remaining 2 species, both methods performed equally well (detection probability 1.00). All in all, detection probability with eDNA metabarcoding was 0.97 (CI 0.90–0.99) versus 0.58 (CI 0.50–0.63) with traditional monitoring. This better performance has immediate and very practical consequences. To achieve the same detection probability obtained with a

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single eDNA sampling event, four successive traditional surveys would have to be undertaken. In terms of man-hours in the field, eDNA metabarcoding appears as an extremely parsimonious biodiversity assessment tool and this could still be enhanced in a near future (Fig. 1). In the laboratory, costs are unlikely to be prohibitive. Following the authors' protocol and assuming catalogue prices, material costs from extraction to sequencing should be less than 100 USD per site.

Fig. 1 Hydroplane drone-assisted water sampling for eDNA metabarcoding. Drones are a promising way to collect more and better samples with even less effort. They can continuously filter water over an entire body of water and access zones otherwise difficult to sample. This may enhance the detection probability of rare species. This drone is double-hulled and the outer hull is disposable, which minimizes the risk of water body cross-contamination. Photograph credit: Alice Valentini and Tony Dejean.

Transferability to other ecosystems and technical developments

The authors make a convincing case for eDNA metabarcoding as a robust, high-throughput tool for molecular ecology and conservation biology. Yet, a number of questions remain open. We will briefly elaborate on two of those. First, is eDNA metabarcoding transferable to any ecosystem? Up to now, eDNA approaches have almost only been applied to temperate areas (including this study; Fig. 2). Animal biodiversity, however, follows a latitudinal gradient which culminates in tropical areas. These regions are both a natural playground for biodiversity research and a major stake for conservation biology. So, could eDNA metabarcoding be employed there? Tropical and temperate environments differ in many ways, and this could affect the outcome of eDNA studies in tropical regions. For instance, higher freshwater temperatures could accelerate DNA decay. On the contrary, higher population densities may result in more intense DNA shedding. Higher species richness itself may come with challenges. Tropical faunas are poorly represented in sequence databases and are more likely to comprise recently diverged species, and this could jeopardize the assignment of many

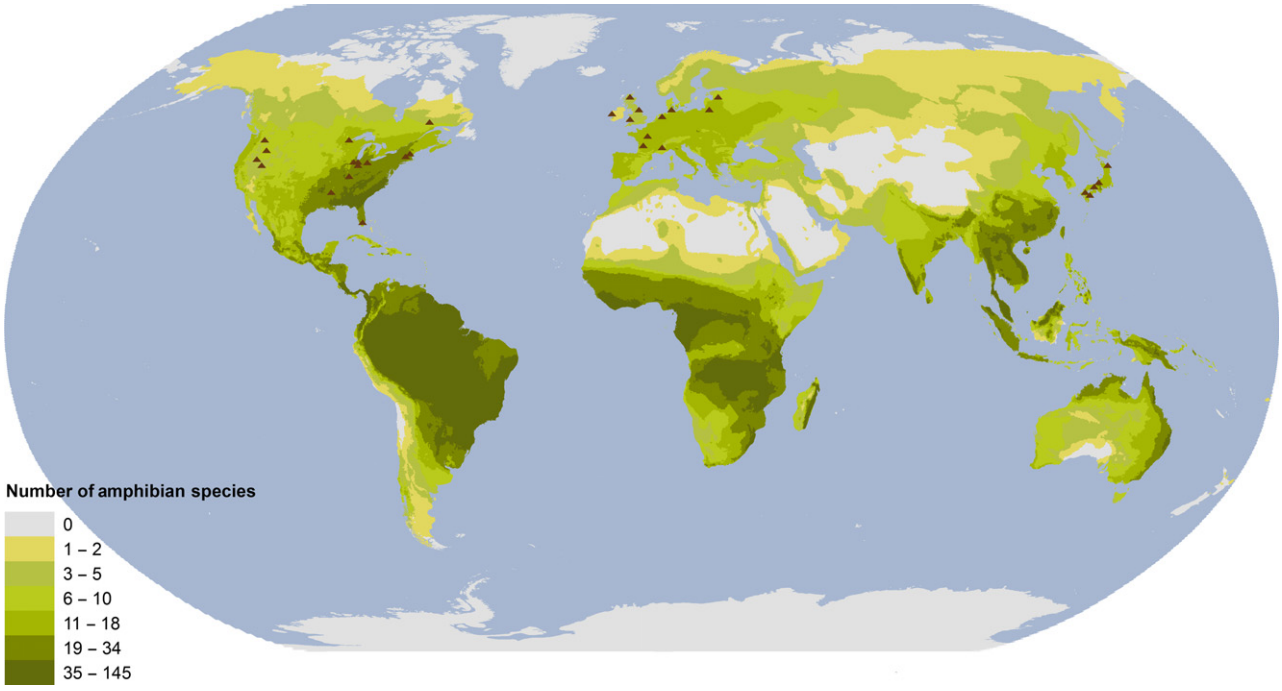

Fig. 2 Environmental DNA dark matter lies under the tropics. Triangles represent sampling locations for all published studies that identified vertebrate DNA from water collected in natural ecosystems (as of November 2015). As an example of vertebrate biodiversity gradient, amphibian species richness is also plotted on this map (according to the IUCN Red List of Threatened Species, 2015). This figure is inspired from Pedersen *et al.* (2015).

sequences. That brings us to our second question: What technical improvements could be imagined? Parallel use of multiple minibarcode systems and/or hybridization capture may enable the production of more sequence information and therefore increase the chances to identify closely related species. Using multiple PCR systems may also shed light on PCR-induced amplification biases. Minimizing and assessing these biases will be crucial to increase individual species detection probability and will become absolutely essential if read counts had to be linked to biomass, as has been proposed for species-specific eDNA approaches (Takahara *et al*. 2012). Taking eDNA metabarcoding on a tropical tour may be an ideal occasion to test their limits and to enhance techniques.

Conclusion

Valentini and colleagues show that eDNA metabarcoding can provide high-quality aquatic biodiversity assessments in temperate ecosystems. As pinpointed by the authors themselves, eDNA metabarcoding can only tell which species are present. This approach should therefore be seen as complementary to conventional methods that will keep bringing crucial additional information about individuals and populations (body size, age structure, etc.). There is no doubt that eDNA metabarcoding offers unprecedented opportunities to scale and speed up aquatic biodiversity assessments. There is also no doubt that setting up eDNA metabarcoding projects will require quite some time and effort. The results presented here however suggest that this initial investment will ultimately pay off – after some hard work, there may be room for (relative) laziness!

References

Boyer F, Mercier C, Bonin A *et al*. (2016) obitools: a unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, **16**, 176–182.

Coissac E, Riaz T, Puillandre N (2012) Bioinformatic challenges for DNA metabarcoding of plants and animals. *Molecular Ecology*, **21**, 1834–1847.

Ficetola GF, Miaud C, Pompanon F, Taberlet P (2008) Species detection using environmental DNA from water samples. *Biology Letters*, **4**, 423–425.

Pedersen MW, Overballe-Petersen S, Ermini L *et al*. (2015) Ancient and modern environmental DNA. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, **370**, 20130383.

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. *Molecular Ecology*, **21**, 1789–1793.

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z (2012) Estimation of fish biomass using environmental DNA. *PLoS ONE*, **7**, e35868.

Valentini A, Taberlet P, Miaud C *et al*. (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, **24**, 929–942.

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