

Detection of aquatic macroinvertebrates by environmental DNA in paddy water

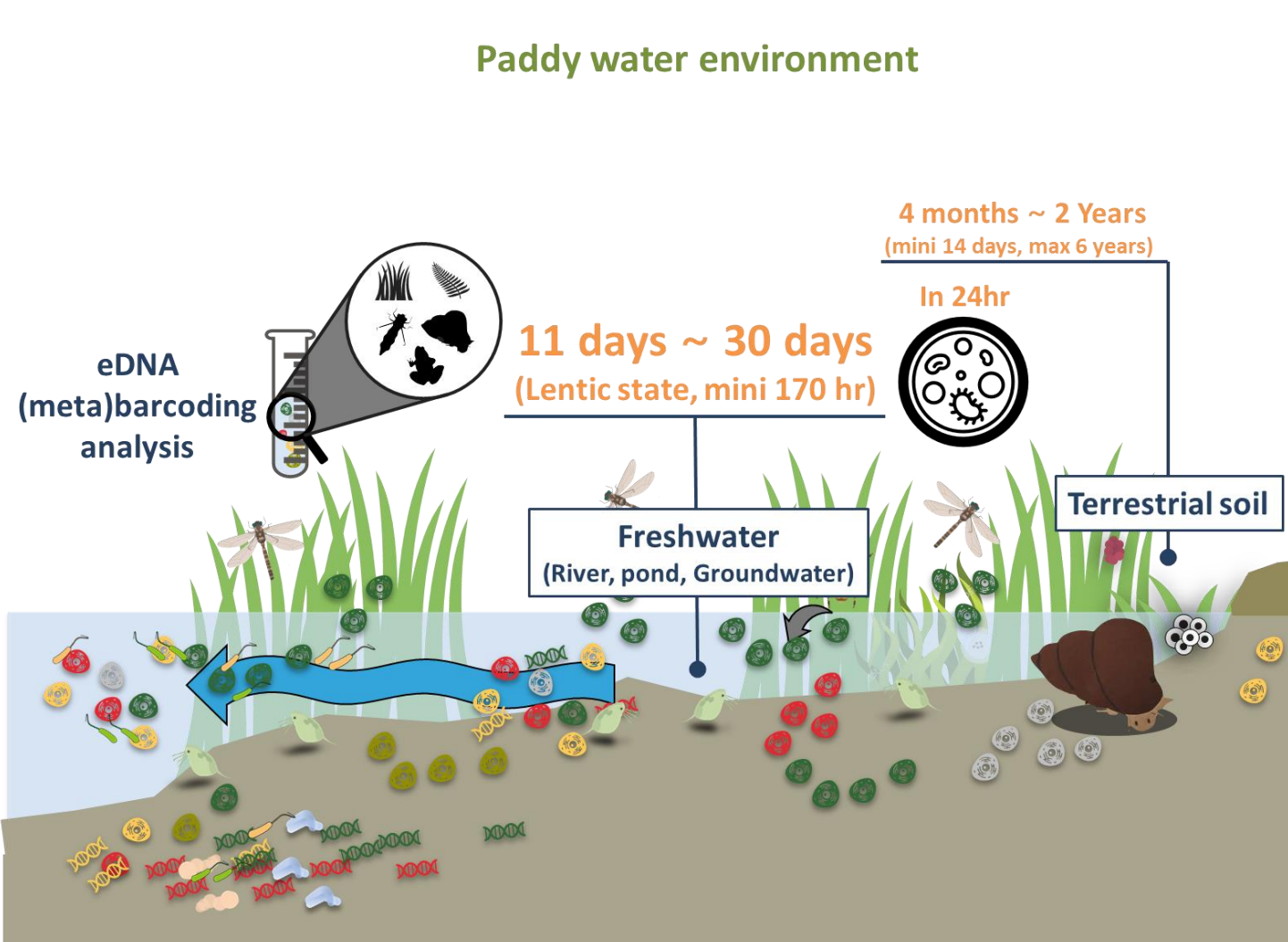
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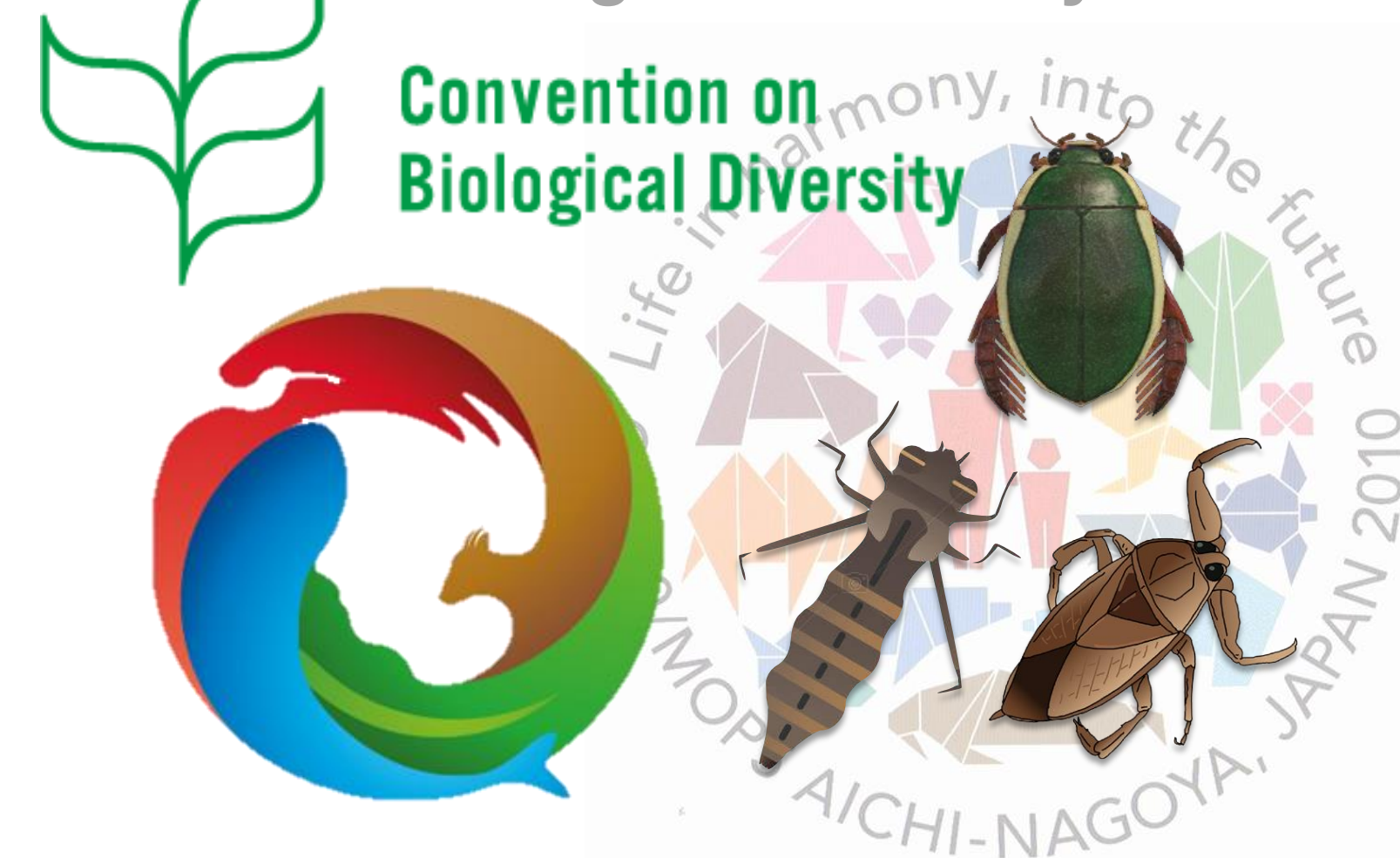
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Main concept

Introduction



Biological Diversity of Paddy field



Biological diversity Is very important for the paddy environment's ecological health



Traditional methods (Trap and Swiping) need fortune and high-level skill for individual capture



Ecology of eDNA in paddy water

Study Site and Methods

-Study site-

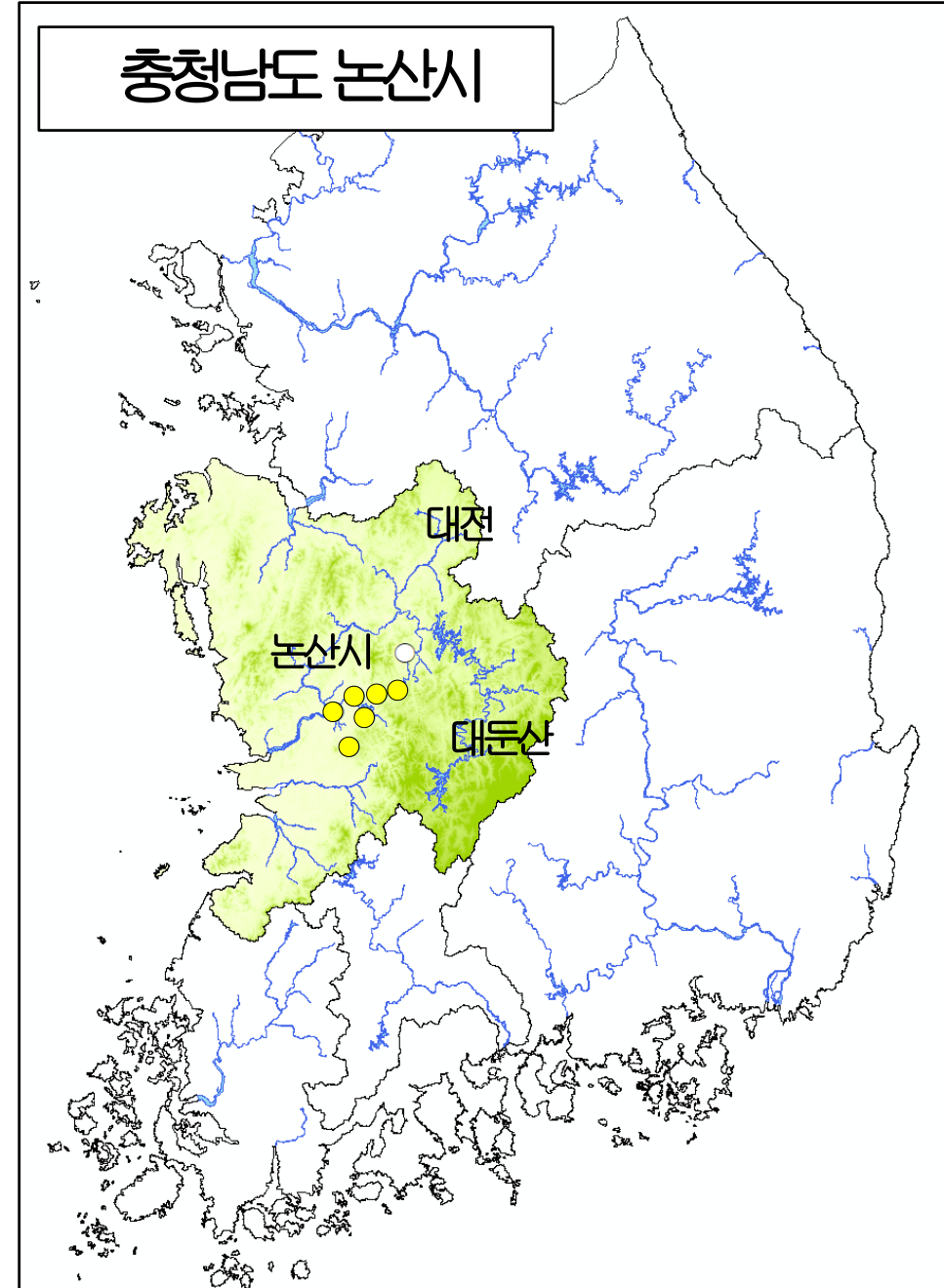
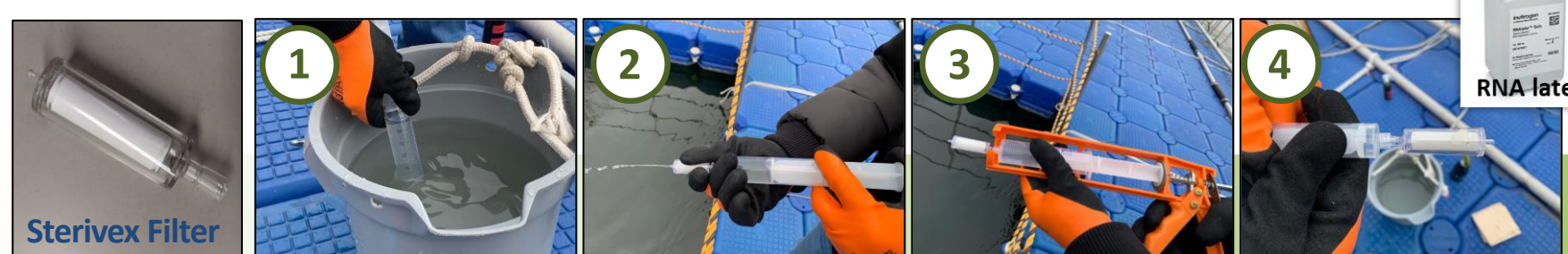


Fig. 1. 6 Sampling paddy sites in Nonsan city were placed in the Chung-Chong Nam-do region.

-Research process-

1. How to sampling eDNA in paddy water

1. Field sampling method (Filtering in Field)



2. Field sampling point (4 point sampling)

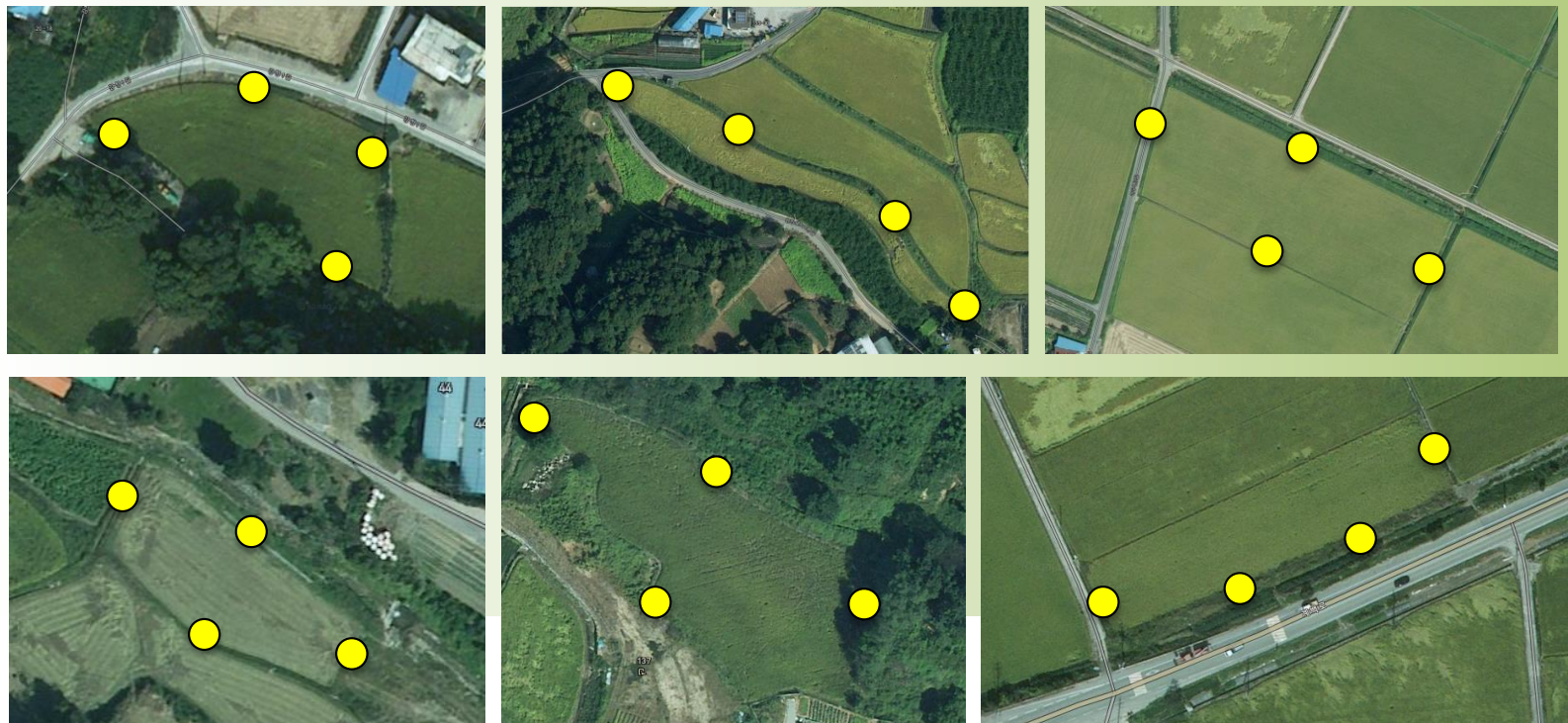


Fig. 2. Sampling point of each paddy field. All sites sampled 4 points in the paddy field. eDNA samples were filtered by Sterivex filter for concentration.

2. eDNA barcoding & meta-barcoding in paddy water

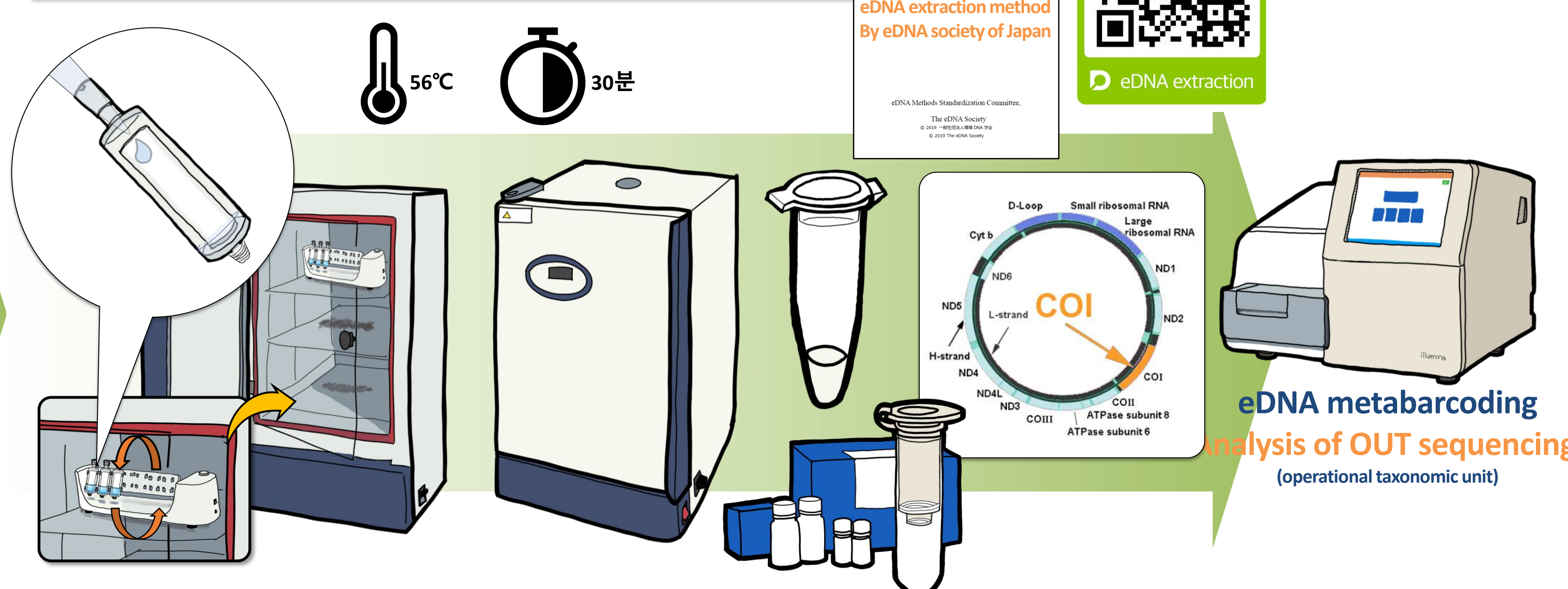


Fig. 3. Process of eDNA in paddy water analysis. eDNA was extracted by "eDNA extraction method" made by eDNA Society of Japan. After extract, all eDNA samples were PCR amplified for COI (cytochrome oxidase subunit 1) gene.

Results and Discussion

eDNA meta-barcoding for COI gene in paddy water

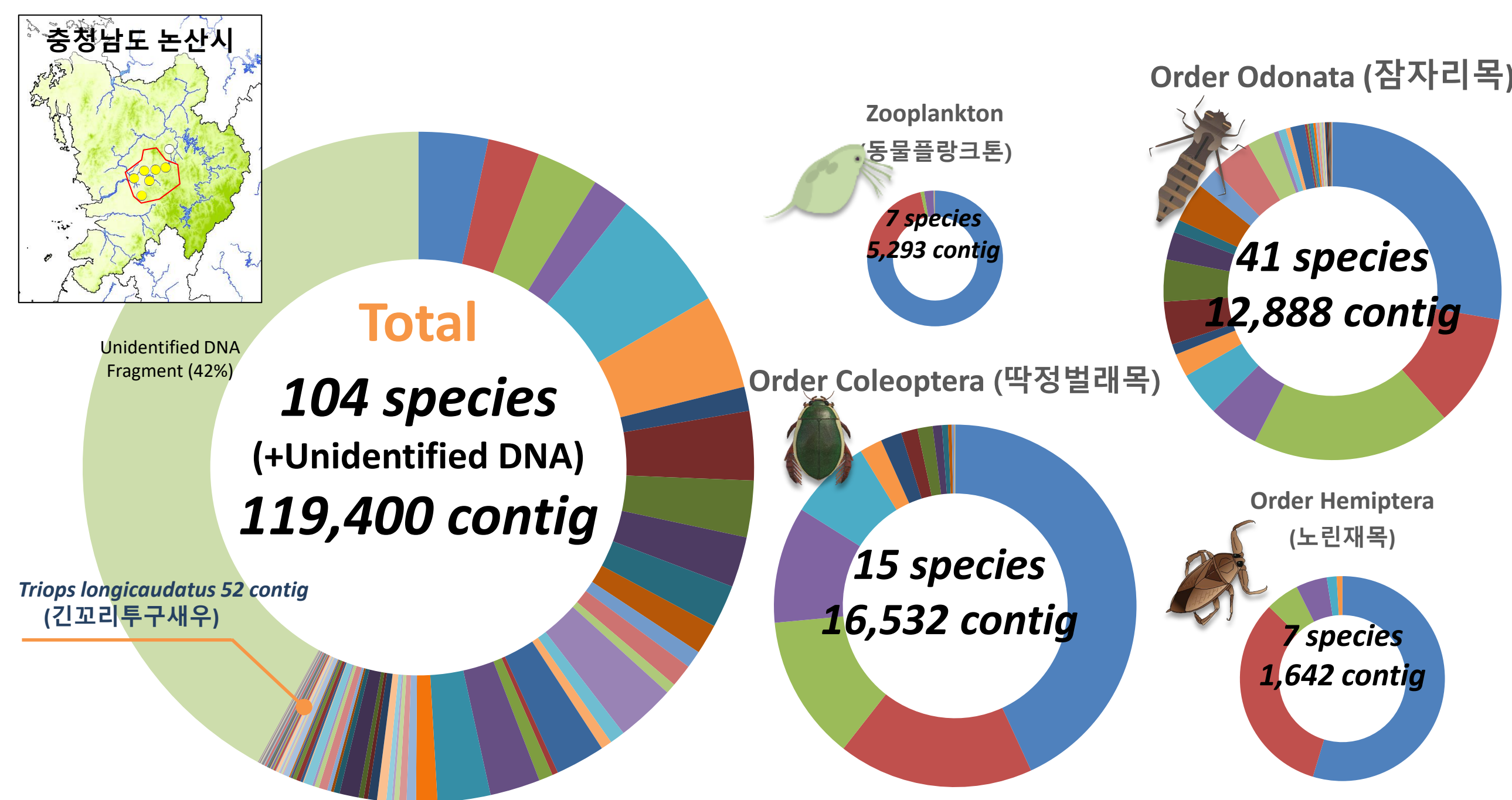


Fig. 4. Results of eDNA meta-barcoding analysis using eDNA existing in 6 paddy field aquatic ecosystems. A total of 104 genes and 119,400 contigs were found in 6 sites. Of these, 42% were not identified as unanalyzed Unidentified DNA. Order of Coleoptera had the highest contig number, but 15 species were found and 41 species were found in the Order of Coleoptera.

Taxon comparison between eDNA and field survey

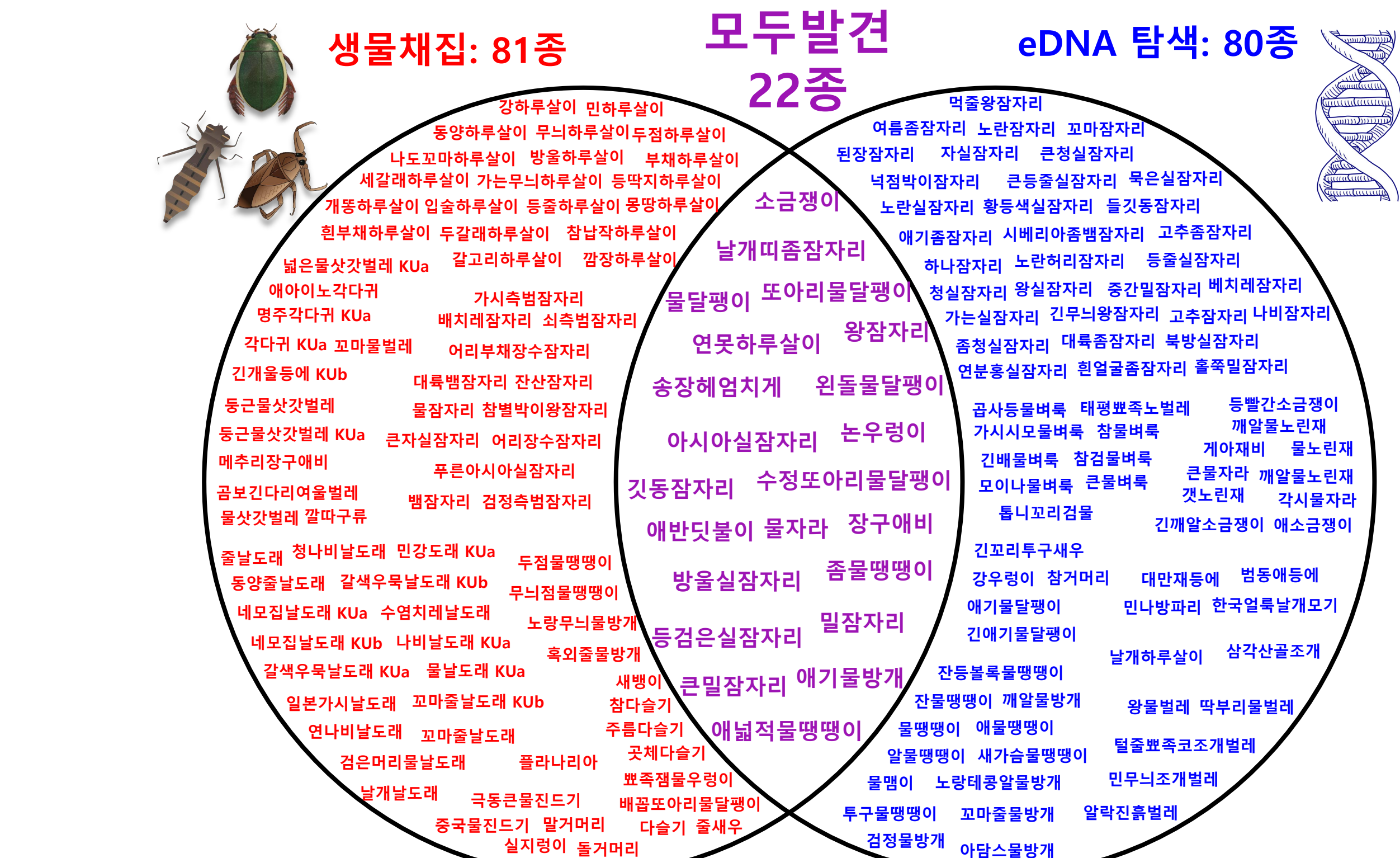


Fig. 6. Intersection taxon list made by eDNA and field survey. Field survey data was collected by Eco-Bank data in NIE(National Institute of Ecology). only 20% of taxon DNA made by meta-barcoding was proved by field survey. 80 species DNA that was only detected in the paddy, might be flowing into the paddy environment from other environments.

Taxa in Aquatic macroinvertebrates community

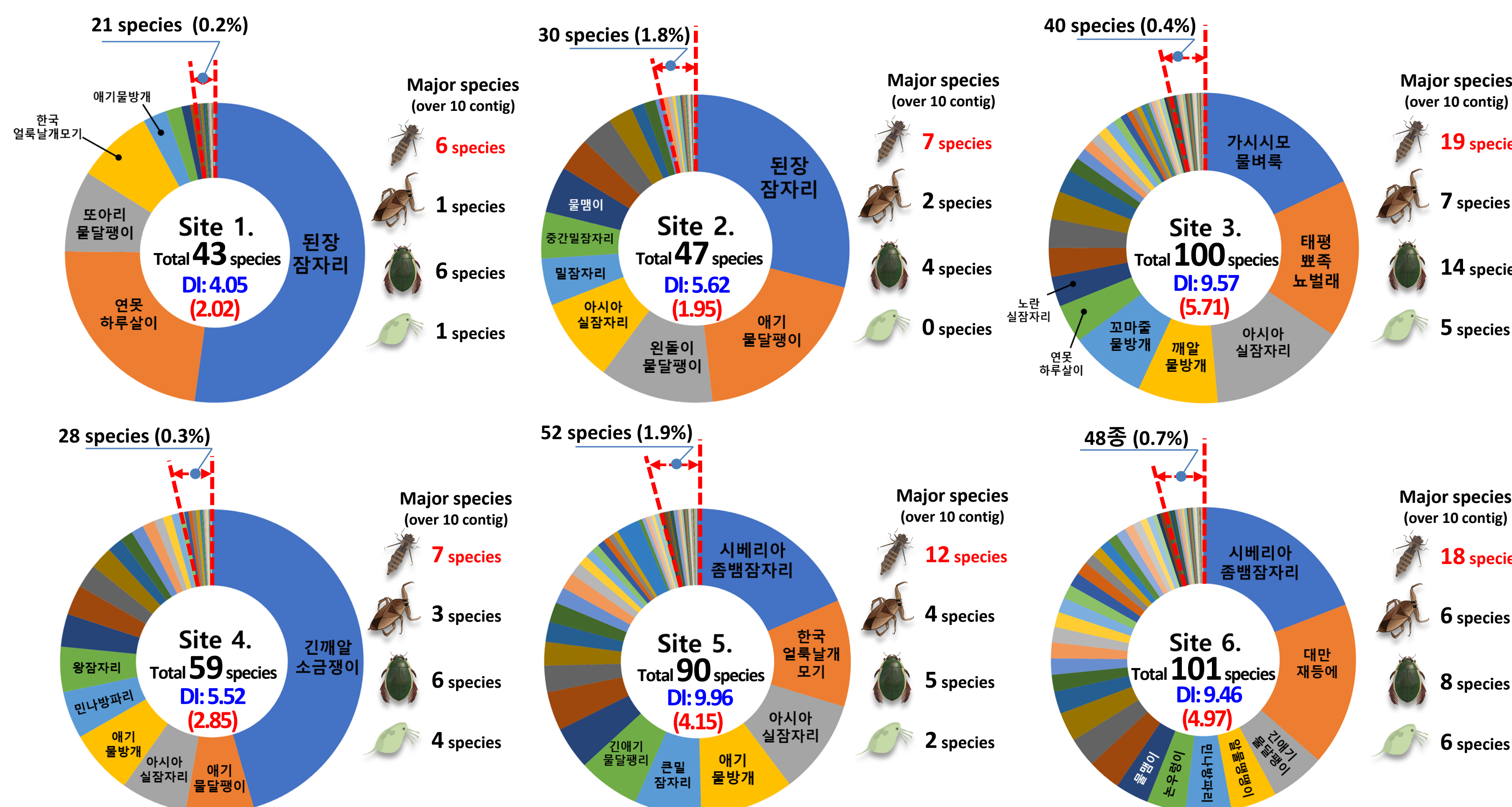


Fig. 5. Effect on change in the ratio and diversity index of rare taxa in arthropod population through eDNA metabarcoding analysis. In all sites, rare taxa (10 contig or less) was less than 2%, and there were a minimum of 21 species and a maximum of 52 species. When these rare taxa were included, the community diversity index (DI) more than doubled (Blue color). More macroinvertebrates species were found in sites adjacent to mountains, and dragonflies were most found in sites.

The detection limit of eDNA concentration to aquatic macroinvertebrates

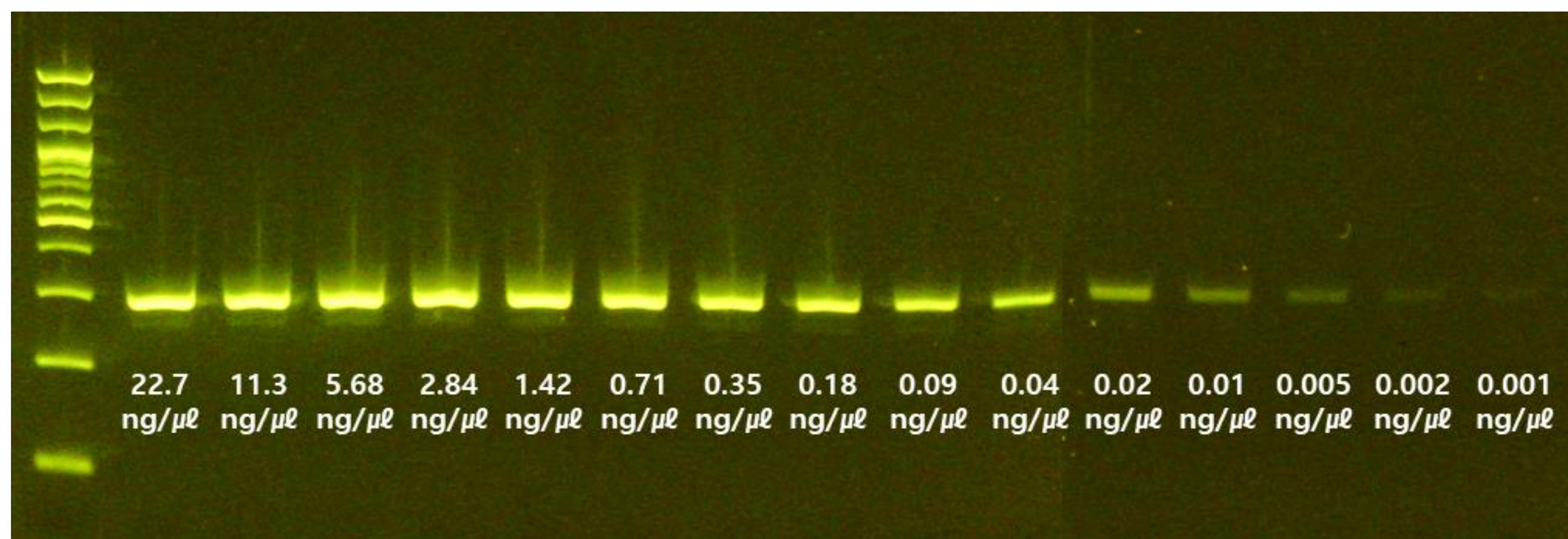


Fig. 7. Detection limit of eDNA samples. In the water column, eDNA can not be detected under 0.001 ng/μL concentration. The ideal eDNA concentration is over 0.18 ng/μL, and when the almost eDNA consists of macroinvertebrate DNA. If eDNA amplicon concentration is low, you need more eDNA amplicon samples.

Conclusions

- eDNA를 이용한 논 수생태계 생물 다양성 탐색은 전통적인 생물 채집 방법보다 다양한 생물 종 탐색이 가능함
- 상대비용 2% 이하의 Rare species를 포함하였을 때 생물의 종다양성이 매우 크게 증가하였으며, 이는 기존 탐색법에서 탐색이 어려운 멸종위기종 등을 탐색하는데 매우 큰 도움이 될 것으로 판단됨
- 하지만 수서 곤충의 유전자가 발견된다 하더라도 이것이 실제 서식하는 생물의 유전자인지 정확하게 판단하기 어려움 (새들의 외부 먹이활동 후 배변을 통해 외부 eDNA가 논 수생태계로 유입될 수 있음. Ex: 시베리아좀뱀잠자리)
- 이외에도 대상생물의 유전자 염기서열 다양성, Primer 염기서열 디자인 문제, Reference 염기서열의 부재 등 실제 현장조사와 일치하지 않는 간극이 발생할 수 있기 때문에 eDNA 탐색결과에 대한 실제 조사검증이 반드시 필요함

Reference

- Matthew A. Barnes and Cameron R. Turner. 2016. The ecology of environmental DNA and implications for conservation genetics. Conservation Genetics (17:1 – 17), DOI 10.1007/s10592-015-0775-4.
- Jelger Herder, Alice Valentini, Eva Bellemain, Tony Dejean, Jeroen van Delft, Philip Francis Thomsen, and Pierre Taberlet. 2014. Environmental DNA - a review of the possible applications for the detection of (invasive) species.. RAVAOON Technical Report, DOI:10.13140/RG.2.1.4002.1208