Strong small-scale differentiation but no cryptic species within the two isopod species *Asellus aquaticus* and *Proasellus coxalis* in a restored urban river system (Emscher, Germany)

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Abstract

Worldwide, humans have strongly altered river networks. Key changes resulted in modified hydromorphology, poor habitat quality and availability, migration barriers, and pollution. Restoration measures aim at mitigating anthropogenic stressors and to restore connectivity, but the biological success of the measures is not guaranteed. Analyzing genetic diversity and metapopulation structure of target species in the river network with genetic markers can help understanding recolonization processes and identifying persisting gene flow barriers. Here, we studied the population genetic structure of two pollution-tolerant detritivorous isopod species in the former heavily degraded and polluted, but now mostly restored Emscher catchment in Germany: the native *Asellus aquaticus* and the non-native *Proasellus coxalis.* For both species, we analyzed mitochondrial cytochrome c oxidase I (COI) gene sequences and nuclear genome-wide single nucleotide polymorphism (SNP) data. Surprisingly, we found a strong metapopulation structure for both species with several isolated populations on a small-scale of few kilometers, but a still high genetic diversity, especially in the COI gene. Both species consisted of two known possible cryptic species, while our SNP data showed, that they represent only one species, each. This highlights the importance of integrating high-resolution nuclear markers into species identification, because species diversity may otherwise be greatly overestimated. While we could identify some migration barriers and found indication for passive dispersal by birds or humans, these factors could not fully explain metapopulation structure, suggesting that also other drivers, such as isolation by adaptation, priority effects or biotic interactions play a role in shaping the local metapopulation structure.

Introduction

As the lowest-lying areas of the landscape, rivers and streams integrate the effects of land-use change and are therefore especially sensitive to and strongly impacted by the changes associated with the urbanization of catchments (Bernhardt and Palmer, 2007). To fit in the urban structure, the original riparian vegetation has often been replaced by impervious surfaces such as asphalt or concrete, thus increasing the surface run-off and substance-load entering urban streams (Bernhardt and Palmer, 2007; Gillmann et al., 2023). For centuries, urban streams have been regulated and modified into mostly straight channels to increase discharge (flood prevention) and to limit the area they occupy. Consequently, urban stream hydromorphology has changed widespread and substantially. The increasing awareness of aquatic biodiversity and its value in concert with a wide range of ecosystem services provided by near-natural rivers and floodplains have resulted in numerous river restoration projects (Jähnig et al., 2011) including urban streams (Gillmann et al., 2023; Winking et al., 2014)
(Jähnig et al., 2011) including urban streams (Gillmann et al., 2023; Winking et al., 2014). However, effects of river restoration on benthic invertebrates, which are frequently used to assess the ecological status of rivers and streams, are often minor (e.g. Jähnig et al., 2010; Lorenz and Feld, 2013; Pilotto et al., 2019; Sundermann et al., 2011). Sundermann et al. (2011) found that the initial (successful) recolonization depends on the species pool that is available in the near surroundings (< 5 km) of restored sites. But also the species-specific dispersal capacity may constitute a major determinant of the successful recolonization of restored stream sections (Gillmann et al., 2024; Hughes, 2007; Jähnig et al., 2010; Sundermann et al., 2011; Winking et al., 2014). The dispersal capacity can be estimated from species life history and dispersal traits (e.g., merolimnic vs. hololimnic, active dispersal vs. passive drift) (Li et al., 2018; Sarremejane et al., 2020) and evidence on realized dispersal distances (e.g. Sondermann et al., 2017; Winking et al., 2014). As a source for recolonization of hololimnic, i.e. fully aquatic species, immigration by drift from connected upstream sites is often assumed to be the major pathway (e.g. Hughes, 2007; Winking et al., 2014). However,

while estimated dispersal capacity and time needed to recolonize a restored stream section may correlate, connectivity or gene flow between newly established and old populations is still difficult to predict (e.g. Weiss et al., 2022; Weiss and Leese, 2016). Besides area- and site-specific factors, such as water pollution, in-stream and terrestrial migration barriers or anthropogenic land use, also intra- and interspecific competition and evolutionary adaptation can play a role in shaping realized gene flow (e.g. De Meester et al., 2002; Fraser et al., 2014). However, connectivity between populations after initial recolonization is important to maintain genetic diversity, because in small, isolated populations, genetic diversity is lost due to genetic drift. The loss of genetic diversity can reduce the capacity of populations to adapt to changing environments (Bijlsma and Loeschcke, 2012; Frankham, 2010, 2005; Hughes et al., 2008; Reusch et al., 2005). To better understand the recolonization process and to evaluate the success, it is important to analyze the connectivity among populations and to recognize migration barriers. These aims can be achieved by analyzing high-density genomic markers, such as single nucleotide polymorphisms (SNPs) distributed across the whole genome (e.g. Fuller et al., 2019; Miles et al., 2019). Different genotyping-by-sequencing approaches exist to generate such SNP data, one popular and powerful being double digest restriction site-associated DNA sequencing (ddRAD-seq, Peterson et al., 2012).

One example for a former strongly degraded and polluted river system, is the Emscher catchment located in the “Ruhr Metropolitan Area” (Western Germany), one of the densest urban agglomerations in Europe (Gerner et al., 2018). In a 30-yearlong project starting in 1990, the Emscher and in particular its tributaries have been restored from a highly modified open wastewater channel system with concrete beds into a wastewater-free, partly near-natural stream system with sinuating or semi-meandering river courses and naturally developed riparian vegetation (Gerner et al., 2018; Gillmann et al., 2023). One key species group, important for ecosystem functioning like organic matter decomposition and therefore important to return after restoration, are detritivorous species such as amphipods and isopods. One isopod species, which can quickly recolonize restored stream sections or even persist in

polluted water, is *Asellus aquaticus* (Gillmann et al., 2023). *A. aquaticus* is opportunistic in waters with various physiochemical conditions, has been found to be relatively pollution-tolerant to organic and chemical pollution, resilient to low oxygen levels and fairly high concentrations of heavy metals in various studies (e.g. Basset, 1993; Fraser et al., 1978; Hervant and Malard, 2019; MacNeil et al., 2002; Maltby, 1995; Van Ginneken et al., 2019, 2017). Further, *A. aquaticus* has been found to be a diverse species complex, comprising several potential cryptic species (Sworobowicz et al., 2020, 2015; Verovnik et al., 2005, 2004). Two of these (OTU A and J, sensu Sworobowicz et al., 2015) have been found both with many haplotypes in the Emscher catchment in a study, in which the correlation of intraspecific genetic diversity of benthic invertebrates with stream degradation was assessed using a metabarcoding approach (Zizka et al., 2020). The high genetic diversity, which has been found in the barcoding fragment of the mitochondrial cytochrome c oxidase I (COI) gene throughout Europe (e.g. Sworobowicz et al., 2020; Verovnik et al., 2005), was mainly explained by survival during glaciations not only in lower latitude refugia, which has been suggested for many terrestrial and freshwater species (e.g. Hewitt, 1999; Taberlet et al., 1998), but also in numerous high latitude refugia (Sworobowicz et al., 2020). However, while many studies focusing on ecological or evolutionary aspects of *A. aquaticus* exist (reviewed in e.g. Lafuente et al., 2021; O'Callaghan et al., 2019), little is known about small-scale connectivity and realized gene flow between populations.

While *A. aquaticus* is a native isopod species in Germany, another non-native species, *Proasellus coxalis*, which is also relatively pollution-tolerant (Spänhoff et al., 2007), occurs in the Emscher catchment. *P. coxalis* is thought to originate from Southern Europe and has been first found in the river Rhine in Germany 1931 (Bernauer and Jansen, 2006). Similar to *A. aquaticus*, it has been found to be a widely distributed, morphologically and genetically variable species group with many described subspecies or molecular operational taxonomic units (MOTUs) (Eme et al., 2018; Ketmaier, 2002; Morvan et al., 2013; Saclier et al., 2024; Stoch et al., 1996), which might be potential cryptic species. While different ecological aspects of the

127 species have been studied (e.g. Basset and Rossi, 1987; Cerfolli and Rossi, 1995; Rossi et
128 al., 1983), less is known about realized gene flow on a small geographic scale, especially in
129 the region, where *P. coxalis* is non-native. A study, analyzing 15 populations of the *P. coxalis*
130 group in coastal and inland areas of Central Italy with allozyme data showed that on smaller
131 geographic scales in the coastal areas, population were isolated by distance, while this pattern
132 was less clear and a higher degree of genetic differentiation was found in the inland area
133 (Ketmaier, 2002). *A. aquaticus* and *P. coxalis* have similar ecological niches and are known to
134 co-occur with no consistent competitive advantage of one species over the other, but a rather
135 complex relationship which can change according to locality and environmental conditions
136 (Kemp et al., 2020).
137 In this study, we aimed to assess and compare small-scale population genomic structure of
138 two freshwater isopod species with different histories, *A. aquaticus* (native) and *P. coxalis*
139 (non-native), in the restored urban river system of the Emscher catchment using two different
140 genetic markers. While COI sequences were generated to identify the MOTUs of both species
141 and to get an overview over the population structure, high-resolution genomic SNPs,
142 generated with ddRAD-seq, are used to test if divergent MOTUs represent different cryptic
143 species and to analyze connectivity and gene flow among populations. With this, our aim was
144 to test the following hypotheses:
145 1. The native species *A. aquaticus* has a high historic (COI) and contemporary (ddRAD)
146 genetic diversity and shows a high connectivity between stream sites within sub-
147 catchments in accordance with an isolation by distance (IBD) pattern.
148 2. The alien species *P. coxalis* shows comparably low genetic diversity (especially in the
149 COI gene) but also high levels of connectivity, with more gene flow between
150 geographically close sites (IBD). This hypothesis is based on the fact that *P. coxalis,*
151 as an alien species, has not been present in the area as long as *A. aquaticus*, but is
152 considered to be similarly pollution-tolerant.
153

Materials and Methods

Sampling

Sampling sites were located in the Emscher catchment, which has a size of 775 km^2. The Emscher is a right tributary to the river Rhine and has several larger tributaries, such as the Berne (catchment area 62 km^2) and the Boye (catchment area 75 km^2). Whereas most of the Boye catchment is already restored, parts of the Berne system still contain wastewater (Fig. 1). The sampling sites were mainly located in tributaries north and south of the Emscher, but also in some streams of adjacent catchments in close distance (Table S1). Most of the sites north of the Emscher belonged to the Boye catchment (23 of 27), while most sites south of the Emscher were located in the Berne catchment (8 of 14, see Tab. S1 for details). Regardless of the catchment, all sites north of the Emscher are abbreviated with BO, while the south sites are abbreviated with BE. The BO sites were mostly congruent with sites sampled in Winking et al. (2014). The sampling was conducted in March and April 2019 and all sites were revisited in March and April 2020. In total, we visited 41 sites, but the focal species of this study, the two isopod species *A. aquaticus* and *P. coxalis*, were only found at 21 of these sites, *P. coxalis* only in Berne and Boye catchment, and *A. aquaticus* additionally at one site belonging to the Lippe catchment. While they were detected at 4 of these sites in sympatry, *A. aquaticus* was exclusively found at 9 sites and *P. coxalis* at 8 sites (Fig. 1, Tab. S1). In 2019 we aimed at sampling 10 isopod specimens per site, while we increased sampling effort in 2020, aiming at 15 specimens per site. However, sampling success differed between years and sites in occurrences as well as in numbers, finally leading to 13 sites for *A. aquaticus* and 12 sites for *P. coxalis* (4 and 5 only one year, respectively; Tab. S1). In both years, the organisms were sampled using sieves and kick-nets, preserved in 96% Ethanol and stored at 4°C until further processing.

Genotyping

Prior to DNA extraction, specimens were morphologically identified. To distinguish between *A. aquaticus* and *P. coxalis*, the identification key of Eiseler (2010) was used. Depending on sampling success, we extracted the DNA of 1 to 16 specimens per site (Tab. S1). In 2019, DNA was extracted following the salt precipitation protocol as described in Weiss and Leese (2016). In 2020, pipetting steps of the extraction were conducted on a Biomek FX^P liquid handling workstation (Beckmann Coulter, Bread, CA, USA) using a modified version of the bead-extraction protocol of the NucleoMag Tissue kit (Macherey-Nagel, Düren, Germany) as described in Buchner et al. (2021). After the extraction, DNA concentration was measured with the Qubit dsDNA BR Assay Kit (Life Technologies; Thermo Fisher Scientific).

For all specimens, the barcoding fragment of the mitochondrial cytochrome c oxidase I (COI) gene was amplified with the standard primers HCO2198 and LCO1490 (Folmer et al., 1994). For the 2019 samples, we used the following PCR protocol: 0.2 µl VWR Taq-Polymerase (5 U/µl), 2.5 µl Key buffer Mg^{2+} free (10x, VWR), 2.5 µl dNTPs (2 mM), 2.5 µl $MgCl_2$ (25 mM), 0.125 µl of each primer (100 µM), 1-3 µl of DNA template, filled up to 25 µl with PCR water. The COI fragment was amplified with the following settings: initial denaturation at 94°C for 2 min; 33-36 cycles of denaturation at 94°C for 20 s, annealing at 46°C for 30 s, and extension at 72°C for 60 s; final extension at 72°C for 5 min. Due to changes in the lab, the 2020 samples were amplified with a different protocol as follows: 5 µl Dream- TaqTM Hot Start Green PCR Master Mix (2x, Thermo Fisher Scientific, Waltham, MA, USA), 0.05 µl of each primer (100 µM), 0.5 µl of DNA template, filled up to 10 µl with PCR water. To increase primer specificity, amplification was performed using a touchdown PCR, where the annealing temperature was decreased by 1°C in each of the first ten cycles: initial denaturation at 95°C for 3 min; 10 cycles of denaturation at 95 °C for 30 s, annealing at 56-46 °C for 30 s, extension at 72°C for 60 s, followed by 40 cycles with the same program with an annealing temperature of 46°C, and a final extension at 72 °C for 5 min. Prior to sequencing, PCR products were purified in both years using 0.5 µl of ExoI (20 U/µl) and 1 µl of FastAP (1 U/µl, both Thermo

Fisher Scientific, Schwerte, Germany). The reaction was incubated for 25 min at 37 °C followed by an inactivation step at 85 °C for 15 min. Bidirectional Sanger sequencing was performed at Eurofins Genomics.

Samples for ddRAD-seq were chosen based on location, year, COI haplotype and DNA concentration, aiming to analyze eight specimens per site and year, if enough individuals had been sampled. In total, we analyzed 280 individuals of both species on 3 sequencing lanes and therefore enhanced specimen numbers to up to 12 specimens per site, where enough individuals had been sampled. This resulted in 164 specimens of *A. aquaticus* and 116 of *P. coxalis*. The ddRAD libraries were generated according to the protocol described in Hupało et al. (2023). Details of the sample preparation for each individual are given in Tab. S2. Depending on the initial DNA concentration up to 600 ng DNA were used for double digestion with the FastDigest restriction enzymes *Csp6*I and *Pst*I (Thermo Fisher Scientific). For choosing most suitable restriction enzymes and calculating adapter quantities for ligation (Peterson et al., 2012), expected cut frequencies and number of fragments were estimated by *in silico* digestion using the script genomecut.pl (Rozenberg, https://github.com/evoeco/radtools/). As no reference genome for the two species was available when designing the study, we used the genomes of two other isopods (*Armadillidium vulgare* and *Ligia exotica* with the NCBI accession numbers LYUU01000000 and BDMT000000000) for the estimation. This resulted in average cut frequencies of 547 bp for *Csp6*I and 7689 bp for *Pst*I. In the PCR, 16 cycles were sufficient for amplifying an adequate number of fragments for sequencing. After measurement, samples were pooled equimolarly into three libraries, aiming at 40 ng of DNA per specimen. The first lane contained 96 specimens of *A. aquaticus*, the second 68 *A. aquaticus* and 29 *P. coxalis*, and the third 88 *P. coxalis* specimens. In addition, the third lane contained 8 ddRAD libraries of *Gammarus pulex* and *G. fossarum* from another study, resulting in 96 specimens per lane.

COI data analysis

The obtained COI sequences were assembled and edited in Geneious Prime 2022.0.2 (https://www.geneious.com) and sequences of each species were aligned with MAFFT 1.4.0 (Katoh and Standley, 2013) as implemented in Geneious with default settings. To check species assignment, sequences were compared with the NCBI database (NCBI Resource Coordinators, 2018). Sequences which were too short or where quality was not sufficient for haplotype determination were only used for species assignment but excluded from further analyses (Tab. S2). The alignments of the remaining sequences were cropped to the length of the shortest sequence per alignment. All following analyses were conducted similarly for both species. First, COI haplotype distances and their frequencies were calculated and visualized as minimum spanning networks (Bandelt et al., 1999) in Popart v.1.7 (Leigh and Bryant, 2015) and colored according to the catchment. To delimitate MOTUs which might represent different cryptic species, we used the ASAP approach (assemble species by automatic partitioning; Puillandre et al., 2021) on the web server, applying the Kimura (K80 ts/tv 2.0) model for computing distances and using otherwise the default settings. For *A. aquaticus*, sequences of main haplotypes from ASAP groups were compared to the studies of Verovnik et al. (2005) and Sworobowicz et al. (2020) to find out to which group or potential cryptic species the specimens from the Emscher catchment belong. For *P. coxalis* no such detailed European phylogeny was found and therefore ASAP group sequences were compared to sequences found in closest proximity to our sampling sites in the Word Asellidae database (WAD, https://gotit.univ-lyon1.fr/en/), using the distribution data based on the study of Saclier et al. (2024) with names of the TH-method for species identification.

To analyze temporal as well as spatial differences between sampling years and locations, haplotype diversity, nucleotide diversity and pairwise F_{ST} values were calculated in Arlequin (v 3.5.2.2, Excoffier and Lischer, 2010) for both years separately as well as combined. Here, only sites were included, where more than 5 specimens have been analyzed. For visualization F_{ST} values and corresponding p-values were plotted in heatmaps using RStudio (Posit team, 2024) and the R packages reshape 2 (Wickham, 2007) and ggplot2 (Wickham, 2016). To evaluate if

the population structure can be explained by geographic distances between sampling sites (isolation by distance, IBD), Mantel tests were conducted with the R-package vegan (Oksanen et al., 2019) using F_{ST} values as genetic distances and waterway distances as geographic distances. Geographic distances were calculated using QGIS v. 2.14.14 (http://qgis.org) with a stream layer provided by the federal state authority LANUV (Gewässerstationierungskarte des Landes NRW © LANUV NRW (2013)). As only few sites were successfully sampled in the Berne catchment for both species, Mantel tests were also conducted using only sites from the Boye system and both tests were plotted together in an IBD plot using the R graphics package (v.4.4.0; R Core Team, 2024). Further, maps for visualization of sampling sites and haplotype distribution were generated with the same stream layer as above in QGIS v.2.14.14 (http://qgis.org) and edited with Adobe illustrator 2024.

ddRAD-seq data analysis

Pre-processing of the three ddRAD-seq libraries was performed similar to Weiss et al. (2018) including demultiplexing and removing of PCR duplicates. To identify loci and genotypes, denovo_mao.pl of Stacks v.1.34 (Catchen et al., 2013) was used. Similar to Hupało et al. (2023), the Stacks pipeline was run with eight different parameter settings according to the guidelines in Paris et al. (2017) to identify optimal parameter settings for each species. The following analyses were executed using the workflow management tool Snakemake (Köster and Rahmann, 2012). The workflow contained stacks2fasta.pl (Macher et al., 2015) and several R and python scripts for data reformatting, filtering and population genetic analyses same as in Weiss et al. (2018). For the subsequent analyses, we used the following general filtering settings: maximum number of single nucleotide polymorphisms (SNPs) per locus was 12, of which only one was subsequently used; minor allele frequency of 1%, a locus had to be present in 90% of the individuals to remain in the dataset. Further, specimens with >40% missing data were excluded from final analyses. Evaluation of Stacks parameter settings was performed by calculating basic population statistics, such as observed heterozygosity (H_O),

observed gene diversity (H_S), overall gene diversity (H_T) and overall F_{ST} and F_{IS} were calculated using hierfstat (Goudet, 2005) in R v.4.4.0 (R Core Team, 2024). To identify genetic clusters, principal component analyses (PCAs; Patterson et al., 2006) were performed and individual ancestry coefficients were estimated based on sparse non-negative matrix factorization algorithms (sNMF; Frichot et al., 2014) using the R-package LEA (Frichot and François, 2015). In the sNMF analysis, the number of clusters was varied between one and 15 with 40 replicates and 500000 iterations per replicate. For selecting the most probable number of clusters (K), cross-entropy values were compared. To analyze temporal and spatial differentiation, pairwise F_{ST} values (after Weir and Cockerham, 1984) between sites (for both years separately and combined) were calculated and significance was tested by bootstrapping over loci (1000 replicates, 0.025/0.975 confidence intervals) with the R-package hierfstat using only sampling sites with > 5 specimens. To test for IBD, the same analyses as described for the COI data were conducted. The divMigrate function (Sundqvist et al., 2016) of the R-package diveRsity (Keenan et al., 2013) was used to assess directional relative migration rates and to detect asymmetries in gene flow using the measure D (Jost, 2008) for sites with > 5 specimens. Genetic diversity was estimated by calculating H_O and allelic richness (AR) at each population (for both years separately and combined) using the same R-package. Further, Neighbor-net networks (Bryant and Moulton, 2004) were calculated using SplitsTree v. 4.14.5 (Huson and Bryant, 2006).

Results

Population structure of *Asellus aquaticus*

In total, we extracted DNA from 215 specimens of *A. aquaticus*, which were found at 13 of the 41 sampling sites, including nine sites at which they were detected in both years (Tab. S1). For all 215 specimens, the COI sequence data verified the species identification. However, some of the sequences were too short or quality was insufficient to determine haplotypes. The

316 resulting final COI alignment included 199 sequences, had a length of 562 bp and contained
317 89 variable sites, of which one was a non-synonymous substitution. In total, we detected 19
318 haplotypes (H1 – H19), of which two had a frequency of > 15%, 14 had a frequency of < 5%
319 and 10 only occurred at one sampling site, each (Tab. S3). Most of these private haplotypes
320 had a frequency of < 3% with the exception of H13 at site BO24 (8%). The partition with the
321 best ASAP score indicated two potential cryptic species (Tab. S4), which could be assigned to
322 the widely distributed OTU A (Sworobowicz et al., 2020; or “Central Europe” group in Verovnik
323 et al., 2005) and to OTU J (or “Trans-Alpine” group), respectively. Most of the specimens from
324 the Emscher catchment belonged to OTU A (15 haplotypes), while OTU J contained only 4
325 haplotypes (H16-19) and was separated by at least 61 mutations (H5 to H16) from OTU A
326 (Fig. 2A). Specimens with haplotypes from both groups were found in each of the three
327 catchments (Fig. 2A and 2B).
328 When considering the haplotype distribution in more detail (Fig. 2B), a generally high haplotype
329 diversity per site becomes evident. Haplotype diversity ranged between 0.404 and 0.824 with
330 a mean of 0.616 and nucleotide diversity between 0.001 and 0.047 with a mean of 0.015 (Tab.
331 S5). With respect to population structure, we found populations from near-natural sites in the
332 northern Boye catchment (BO24, BO27) to be significantly differentiated from all other sites
333 (Fig. S1A), containing only private and rare (shared with other populations but frequency <
334 10 %), but none of the main haplotypes. All other populations of the Boye catchment were
335 significantly differentiated from some, but not all populations in both, Boye and Berne
336 catchment. An IBD pattern was only detected when regarding solely distances within the Boye
337 catchment ($r = 0.563$, $p = 0.001$), but not when including also distances to Berne populations
338 ($r = -0.008$, $p = 0.46$; Fig. S2A). Allele frequencies were stable through time, as indicated by
339 the lack of significant differentiation between populations per individual sites across years (Fig.
340 S1A).
341 To investigate the population structure at higher resolution, we generated ddRAD data for 164
342 specimens. Depending on the Stacks settings, we obtained between 2444 and 2806 loci when

including all specimens. As all basic population genetic statistics were similar between settings, we decided to further use the Stacks setting, which resulted in the highest number of loci (Tab. S6). Excluding specimens with > 40% missing data (4 specimens) resulted in 3302 loci, which were used for all further analyses. To get a first overview of the population structure and check if the ASAP COI OTUs represent cryptic species, we conducted a PCA. Here, the first 12 axes were significant, with the first three explaining 5.4, 2.4 and 1.8% of the variation, respectively. As visible in Fig. 2C, most of the individuals from one sampling site clustered closely together regardless of the corresponding haplotype group, indicating the presences of only one species in the area. This was also supported by the neighbor net, where one big group was visible with specimens from the same site clustering mostly together (Fig. S3). Additional to the PCA, the fine scale population structure is also visible in the sNMF analysis, where five clusters best represented the population structure according to the cross-entropy criterion (Fig. S4B). When displaying the sNMF plot for k=5 on the map (Fig. 2D), five separated populations are visible. Here, two of the populations from the northern near-natural sites of the Boye catchment (BO24 and BO27) are each separated from all other populations (Fig. 2C), also corresponding with the PCA analysis. Similarly differentiated from all, is population BO07, which is located in a restored stream further downstream in the Boye catchment. Another cluster is mainly present in populations BO15 and BO16, both located in close proximity (1 km distance) in the same stream. In the PCA these two populations are not distinguishable from the three Berne populations regarding the first two PCA axes (Fig. 2C), but separated with the third axis (Fig. S4A). In the sNMF analysis, the three Berne populations (BE29, BE30, BE31) form the fifth cluster. The remaining five populations, including BO02 which was located in a neighboring catchment, were a mix of all five clusters, but in different proportions, also visible in the PCA where they grouped together. Nevertheless, nearly all F_{ST} values indicated a significant differentiation in a range between 0.006 and 0.189 (considering only comparisons for n > 5), except comparisons between BE30 and both, BE29 and BE31, but including the comparison of BE29 and BE31 (Fig. S1B). Further, low F_{ST} values were found

between the Berne and some Boye populations (i.e. BO16, BO15, BO00 and BO25). Similar to the COI data, an IBD pattern was only found when considering solely comparisons within the Boye catchment ($r = 0.4$, $p = 0.031$; Fig. S2C), because in some cases, differentiation was lower between populations located in different catchments, than within the Boye catchment. When comparing specimens sampled at the same location but in different years, none of the comparisons indicated a significant differentiation. Highest gene flow was detected between the three Berne populations and between BO15 and BO16 in the DivMigrate analysis (Fig. S5A). Further, none of the pairwise comparisons of directional gene flow between sites was significantly asymmetric. Genetic diversity in terms of allelic richness ranged between 1.38 and 1.47 (mean: 1.43), while observed heterozygosity was relatively constant between sites, ranging between 0.11 and 0.13 (mean: 0.12; Tab. S5).

Population structure of *Proasellus coxalis*

P. coxalis was detected at 12 sites, including five sites at which specimens of the species were only found in one year (Tab. S1). At the four sites, where both species occurred in syntopy, *P. coxalis* was always less frequent than *A. aquaticus*. In total, we extracted DNA for 142 specimens and could use all COI sequences to verify the morphological species identification. Due to insufficient quality, some of the sequences were excluded from the final analyses, resulting in 124 sequences in the final alignment. The final alignment had a length of 520 bp and contained 40 variable sites of which 3 were non-synonymous substitutions. In contrast to *A. aquaticus*, only six different haplotypes (H1 – 6) were detected, of which three had a frequency of < 5% and were private for different sampling sites (Tab. S7). The best two partitions of the ASAP approach indicated three potential cryptic species, of which two were assigned to MOTU 240 (named MOTU 240A and 240B hereafter) and the third was assigned to MOTU 241 (both names from WAD, HT approach, Saclier et al., 2024). Most of the specimens belonged to MOTU 240A, which contained the haplotypes H1 and H2 and was present in both, Boye and Berne catchment (Fig. 3A). MOTU 240B only consisted of haplotype

H3 (4 specimens, site BO27). MOTU 240A and B were separated by six mutations, while MOTU 241 was separated by 30 mutations from MOTU 240A and 36 from MOTU 240B. MOTU 241 consisted of haplotypes H4 – H6 and was present in 29% of the specimens mainly in the Boye system, but H4 also occurred at site BE20 (Fig. 3B). Similar to *A. aquaticus*, populations in the north of the Boye system (BO26, BO27 and BO31) were significantly differentiated from all other populations (Fig. S1C), while Berne populations were not significantly differentiated from the southern Boye populations. At five sites, less than five individuals were found and these populations were therefore excluded from the F_{ST} analysis. Both, for the full dataset and for the Boye catchment alone, no IBD pattern was detected (Fig. S2B). When considering only the Boye catchment, a similar trend as for *A. aquaticus* was visible, but too few comparisons remained to detect a significant correlation ($r = 0.441$, $p = 0.133$). Similar to *A. aquaticus*, no temporal differentiation was detected in the COI data (Fig. S1C). Genetic diversity was lower in comparison to *A. aquaticus* with a mean haplotype of 0.187 and a mean nucleotide diversity of 0.008 (Tab. S5). Haplotype diversity ranged between 0 and 0.268 with the exception of site BO27 where both species occurred together and where highest haplotype (0.667) and nucleotide diversity (0.0238) were found.

To analyze fine scale population structure, ddRAD libraries were generated for 116 specimens. Depending on the Stacks settings, 9848 to 11764 loci were retained (Tab. S6) when all specimens were included. Because all basic population genetic statistics were similar between settings, we decided to use the stacks setting with the most loci (“m3 M3 N5 n4”) for the final analysis. Excluding specimens with > 40% missing data (1 specimen), resulted in 12186 loci which were used for all further analyses. To check if the ASAP COI MOTUs represent cryptic species and analyze the population structure, we conducted a PCA. The first 9 axes were significant, with the first three explaining 5.5, 4.3 and 4.1% of the variation, respectively. When plotting the first to axes (Fig. 3C), clustering was not driven by the haplotype group, but the sampling site, as all specimens from one sampling site clustered together regardless of the ASAP group. This was also visible in the Neighbor net, where

424 specimens from most of the sites were clearly separated, but not structured according to the
425 haplotype groups (Fig. S6). The generally strong population structure is also visible in the
426 sNMF analysis, where five clusters best represented the population structure according to the
427 cross-entropy criterion (Fig. S4C). On the map, five clearly separated groups are visible (Fig.
428 3D). Similar to *A. aquaticus*, two populations from the northernmost near-natural streams, i.e.
429 BO26 and BO31, were clearly separated from all other populations. However, this does not
430 include BO27, which was strongly differentiated for *A. aquaticus*, but clusters together with a
431 big group of more downstream populations (BO23, BO20, BO21, BO25 and BO07) for *P.*
432 *coxalis*. The same is true for the syntopic population BO07, which was differentiated for *A.*
433 *aquaticus*, but belonged to the main cluster for *P. coxalis*. For both species, BO25 was not as
434 differentiated as the other near-natural upstream populations. For *P. coxalis*, only one
435 specimen was found at this site, which was however clearly differentiated from the close
436 upstream site BO26 (1.87 km). Similar to *A. aquaticus*, specimens from the oldest restored
437 sites in the Vorthbach (BO17 and BO16 for *P. coxalis*) formed a distinct cluster. However, the
438 single specimen found at BO16 (0.3 km downstream of BO17) was separated from BO17 in
439 the PCA. The last cluster is formed by individuals from the Berne catchment (BE20 and BE21),
440 which were, in contrast to indications from the COI analysis, strongly isolated. The general
441 strong small-scale differentiation between sampling sites is also indicated by the F_{ST} values,
442 which were all significant (considering only comparisons for $n > 5$) and ranged from 0.003 to
443 0.197 (Fig. S1D). While most of the populations from the big group from the sNMF analysis
444 were too small to reliably calculate F_{ST} values, the included comparison between BO23 and
445 BO27 also indicated significant differentiation, but showed the second lowest value (0.093)
446 after the comparison of the two Berne populations (BE20 and BE21; 0.003). In contrast to the
447 COI data and both data sets for *A. aquaticus*, an IBD pattern for the whole dataset was
448 detected for *P. coxalis* ($r = 0.558$, $p = 0.003$; Fig. S2D). When considering only distances from
449 the Boye catchment, a similar but non-significant trend was found as for the COI data
450 ($r = 0.408$, $p = 0.2$). In contrast to *A. aquaticus,* low but significant differentiation was indicated

for three of the temporal comparisons (BE21, BO17 and BO26), while the others were not significantly differentiated (Fig. S1D). Highest gene flow was detected between the two Berne populations in the DivMigrate analysis (Fig. S5B), but was low between all other populations (only comparisons for n > 5). Further, no significantly asymmetric gene flow was detected. Allelic richness was similar to *A. aquaticus*, ranging from 1.4 to 1.48 (mean: 1.44), while observed heterozygosity was similar between all sites, ranging between 0.13 and 0.14 (mean: 0.14; Tab. S5).

Discussion

In this study, we used a population genomic approach to quantify patterns of genetic diversity and population structure of two pollution-tolerant isopod species, one native and one non-native species, in a restored river system in a heavily urbanized area. Our first hypothesis postulated the native species *A. aquaticus* to show high genetic diversity as well as a high connectivity between sampling sites following an IBD pattern. However, while we found a high COI haplotype and nucleotide diversity, nuclear genetic diversity was not particularly high and connectivity between most of the sampling sites was lower than expected according to the hypothesis.

As presumed from Zizka et al. (2020), we found both, OTU A and OTU J in the Emscher catchment. Otherwise, OTU J had only been recorded in Southern Germany, France, Italy, Slovenia and Croatia (“Trans-Alpine” Group), but sometimes in sympatry with OTU A, which had the widest distribution range of all discovered OTUs (Sworobowicz et al., 2015). Additional to the mitochondrial COI gene, Sworobowicz et al. (2015) analyzed the nuclear 28S rDNA gene, finding a mito-nuclear discordance pattern as only few of the mitochondrial groups were supported by the nuclear data, including OTUs A and J, which shared two 28S haplotypes. This pattern was interpreted primarily as a result of incomplete sorting of nuclear lineages, or in some cases, as a result of introgression of formerly isolated peripatric mitochondrial

478 lineages. On the contrary, our data suggest that in case of *A. aquaticus*, COI may greatly
479 overestimate species diversity, while the nuclear 28S data may be more reliable for species
480 delimitation. An extremely high intraspecific diversity in the COI gene has been found to be a
481 general pattern for terrestrial and freshwater isopods and interpreted to be the result of
482 phylogeographic events, *Wolbachia* infections, atypical mitochondrial DNAs, heteroplasmy or
483 a combination of these factors, instead of an indication for the presence of multiple cryptic
484 species (Raupach et al., 2022). In our study, specimens of the less frequent OTU J always
485 occurred in sympatry with OTU A and were not differentiated in the nuclear SNP data, but
486 clustered together with the other specimens from the same sampling site. As the distribution
487 of OTU J is otherwise trans-alpine and the COI diversity was much lower in the area than of
488 the dominant lineage OTU A, it can be assumed, that OTU J colonized the Emscher area only
489 later from Southern Europe, probably similar to *P. coxalis*, which also has its origin there. Both
490 OTUs have also been identified in the metabarcoding-study (Zizka et al., 2020), indicating that
491 such an approach is suitable to detect haplotype diversity.
492 While we found a relatively high genetic diversity with regard to mitochondrial COI haplotype
493 and nucleotide diversity, nuclear diversity in terms of allelic richness (AR) was not particularly
494 high and H_O was relatively low at all sampling sites. AR was comparable to other
495 macroinvertebrate species in a less degraded, but strongly fragmented area relatively close to
496 the Emscher catchment (Ruhr-catchment; Weiss et al., 2022). The lower nuclear diversity is
497 more in accordance with the general strong, but unexpected population structure. Because *A.*
498 *aquaticus* is relatively pollution-tolerant, was present in the streams directly after restoration
499 and might even have survived harsh conditions in polluted waters (Gillmann et al., 2023), we
500 expected high connectivity between sites and accordingly a low differentiation. At a few
501 neighboring sites, e.g. at sites in the Berne system, where distance between sites was less
502 than 1.8 km, high gene flow was detected in accordance with the hypothesis. However, at the
503 neighboring sites in the Boye system (i.e. BO15 & BO16, 1 km apart), gene flow was already
504 reduced, even though it was still high in comparison to all other sites within the Boye system,

505 which had a distance of 1.9 km to 10.5 km from each other. In accordance with an IBD pattern,
506 populations within the Boye catchment were significantly differentiated by distance. However,
507 this was not the case when populations of the Berne catchment were included, because
508 differentiation between Berne and Boye populations was sometimes lower and estimated
509 migration rates higher than within the Boye catchment over shorter waterway distances. The
510 lowest differentiation of Berne populations was found to Vorthbach (BO15&BO16) and Boye
511 (BO00, BO25) populations. However, in-stream migration through Berne, Emscher and Boye,
512 is unlikely, because of the distance and the many still existing migration barriers like
513 wastewater, channelization and water level differences between Boye and Emscher.
514 Therefore, passive over-land dispersal by human activities (e.g. river restorations or monitoring
515 at different sites in the same time frame) or bird-mediated transport, i.e. by specimens being
516 carried in the feathers and/or weeds associated with aquatic birds (Coughlan et al., 2017;
517 Sworobowicz et al., 2020; Verovnik et al., 2005), seems to be the more likely explanation here.
518 In contrast, differentiation between Boye populations was in some cases higher than expected
519 considering the distance between sites, indicating that other additional factors are shaping the
520 genetic structure. Here, two of the populations in the near-natural sites (BO27 and BO24) and
521 three populations from restored sites (BO07 and BO15&BO16, together), each had their own
522 cluster in the sNMF analysis, indicating the presence of migration or gene flow barriers and
523 that restored sites were either recolonized after restoration from an unidentified source or that
524 populations have survived in the polluted parts and remained isolated there. In the region,
525 many small ponds containing *A. aquaticus* populations exist, which were not sampled here,
526 but from which recolonization of stream habitats by passive dispersal via birds could have
527 happened. To analyze the role of this dispersal mechanism in structuring metapopulations, it
528 would be important to also genetically analyze these populations. For two of the populations,
529 BO24 and BO07, potential migration barriers exist, while they were less obvious for the other
530 isolated populations. At the outlet of the Nattbach (BO07) into the Boye, an impassable drop
531 existed, which was only recently (2021) removed. Further, the lower part of the Quaelingsbach

(BO24) is still channelized as it runs directly next to a highway and the middle part falls often dry in summer. For BO27 and BO15&16, no direct barrier could be identified, but also here, parts of the streams sometimes fall dry in summer and the most parts of the Boye were only restored relatively recently. Another potential migration barrier, a pumping station where the water of the Boye is pumped through a pipe because of mining subsidence, exists in the Boye downstream of site BO25. However, this population was not as isolated as would be expected, but clustered together with other population from restored sites, indicating again the importance of passive dispersal. The absence of obvious migration barriers for some populations and the isolation of other populations despite the possibility of passive dispersal, can indicate that in some cases gene flow is restricted despite possible migration. This can occur, when specimens disperse but cannot establish in the new population, which can be explained by either a density-dependent priority effect (Fraser et al., 2014), or according to the monopolization hypothesis by a combination of numerical advantage together with adaptation of the first migrants (De Meester et al., 2002). Here, it might be possible that the individuals from the near-natural sites (BO24, BO27) are less adapted to the conditions in the restored sites than other source populations, which might even have survived in conditions prior to restoration. This could lead to a priority effect of source populations from former polluted sites over populations from restored sites. Priority effects and isolation by adaptation have also been identified in other studies for different species (e.g. Funk et al., 2011; Nosil et al., 2009, 2008; Urban and De Meester, 2009). However, we could not test this hypothesis with our data.

One additional factor, which could have shaped the population apart from distance, migration barriers and adaptation, is competition either between *P. coxalis* and *A. aquaticus* or with the two amphipod species, *G. pulex* and *G. fossarum*, which co-occur in the system at many sites and have a similar ecological niche (Graça et al., 1994a). For the two isopod species, it is difficult to predict which of the species has a competitive advantage, because both species were found to have an advantage over the other in different studies (e.g. Burmeister, 2003; Costantini and Rossi, 1998), or showed potential for niche differentiation when occurring in

559 sympatry (Costantini et al., 2005; Rossi et al., 1983), so competitive relationship could be
560 different according to locality or environmental conditions (Kemp et al., 2020). In our study, we
561 found less *P. coxalis* than *A. aquaticus* specimen and at the four sites, where both species
562 occurred, we always found more *A. aquaticus* specimens. However, at most of the sites, only
563 one of the species was found. Here, *A. aquaticus* was mainly found at permanent sites, while
564 *P. coxalis* was found at temporary sites which often dry out in summer or directly below drying
565 stream sections, indicating that *P. coxalis* can better cope with these conditions. When
566 comparing *G. pulex* with *A. aquaticus*, it was found that *G. pulex* normally dominates in clean
567 water, while in more polluted water, *A. aquaticus* becomes the dominant species (MacNeil et
568 al., 2002; Whitehurst, 1991). Similar observations have also been made in the Emscher
569 catchment, where over time a decrease of *A. aquaticus* was found together with an increase
570 of *G. pulex* (Gillmann et al., 2023). Here, we found co-occurrence of both isopod species and
571 either one or both of the amphipod species at all but one site (BO24). Therefore, it is difficult
572 to understand how the presence of amphipods could have shaped the population structure in
573 the two isopod species, but as water quality should improve more over time, it could be
574 expected, that populations of isopod species will further decline. However, it has not been
575 found, yet, what drives the competitive advantage of *G. pulex* over *A. aquaticus* in cleaner
576 water, except that *G. pulex* is more adapted to higher flow velocities. Until now, there has been
577 no evidence for competition for spatial resources (Graça et al., 1994b) or an indication that
578 food is important in the separation of both species (Graça et al., 1994a). Therefore, continuing
579 coexistence of the four species could still be possible, and co-occurrence and competition
580 dynamics would need to be addressed e.g. in a time series study.
581 Our second hypothesis postulated genetic diversity to be lower in the similarly pollution-tolerant
582 species *P. coxalis*, especially in the COI gene, because it is an alien species, but connectivity
583 to be similarly high as in *A. aquaticus*. In accordance with the hypothesis, we found a much
584 lower haplotype diversity in general (6 vs. 19 haplotypes), but also at all but one of the sampling
585 sites. This can be expected in invasive species, when the number of initial colonists is small,

586 leading to reduced genetic variation in comparison to source populations due to the bottleneck
587 effect and genetic drift (Sakai et al., 2001). However, when multiple introductions occur from
588 different source populations, genetic diversity can also by higher than in any single source
589 population (Sakai et al., 2001). In contrast to haplotype diversity, AR and H_O were not reduced
590 in comparison to *A. aquaticus*, but even slightly higher. To better understand the colonization
591 process of *P. coxalis* from southern Europe, it would be essential to analyze both mitochondrial
592 and nuclear marker in the native distribution range as well as along proposed migration routes.
593 The integration of nuclear markers is especially advisable here, because similar to *A.*
594 *aquaticus*, three OTUs or potential cryptic species were identified for *P. coxalis* with the COI
595 gene and many more were found and deposited in World Asellidae Database (WAD) by
596 different sources and delimitation approaches (Eme et al., 2018; Morvan et al., 2013; Saclier
597 et al., 2024). In Zizka et al. (2020), *P. coxalis* was only detected at one site in the Emscher
598 catchment and all haplotypes belonged to MOTU 240, which was also the dominant MOTU,
599 when combining ABGD groups 240A and 240B, in our study as well as when considering
600 distributions in WAD. Here, MOTU 240 had the broadest distribution, ranging from Spain over
601 France and Italy to Croatia and Bosnia and Herzegovina, with occurrences also in Germany,
602 the Netherlands and Sweden. Even though less abundant, the other OTU detected in the
603 Emscher region, MOTU 241, had a similar southern distribution range from the south of France
604 to Bosnia and Herzegovina with the northernmost occurrence in the east of France close to
605 the German border. Both MOTUs have been found to occur in sympatry. As with OTUs in *A.*
606 *aquaticus*, our data clearly show evidence for only one species in our study area, suggesting
607 also that these MOTUs might in general belong to one species. To delimit species in the broad
608 *P. coxalis* species group, which also includes many subspecies, a Europe wide sampling would
609 be needed. However, our data show how important it would be to integrate nuclear data here
610 to disentangle species in this species complex and to understand invasion routes.
611 As for *A. aquaticus*, we expected high levels of connectivity within Boye and Berne system.
612 However, estimated migration rates between sites were even lower than in *A. aquaticus* except

for the two neighboring sites in the Berne system, which were only 0.8 km apart, but together clearly separated from populations of the Boye catchment. While this stream still contains wastewater in the downstream part, it can be temporarily dry in summer in the restored part, which could explain why we found low but significant differentiation between time points. Further, there are small ponds are located close by, from which recolonization after restoration and also after dry periods can happen for example via aquatic birds. Similarly, also the other sites, where we found differentiation between time points (BO17 and BO26) dry often out in summer, explaining the temporary instability and also partly the isolation of the populations. BO17 is close to the spring of the Vorthbach, where *P. coxalis* can probably survive the dry periods. While otherwise no obvious migration barriers exist further downstream, for both of the other isolated populations in temporary streams (BO26 and BO31), an additional barrier (pumping station) exists downstream of the sites, explaining especially the strong differentiation between BO31 and BO27 detected by both marker systems. The other populations which were located closer to or within the Boye, where less differentiated, belonging mainly to one genetic cluster, including also the one specimen from BO25. This site is located upstream of the Boye pumping station, but the specimen was closer related to the downstream populations BO20/21 than to the upstream population BO26, indicating passive dispersal events similar to *A. aquaticus*. In contrast to *A. aquaticus*, populations at both BO07 and BO27, were not as isolated for *P. coxalis*. At BO27, this indicates again that *P. coxalis* can cope better with the summer droughts, maintaining connectivity with closely located sites (i.e. BO23, BO20/21). However, it is unlikely that *P. coxalis* can actively overcome the instream barrier which separates BO07 from the Boye, indicating passive dispersal mediated by humans or birds.

In contrast to *A. aquaticus*, genetic differentiation in *P. coxalis* was correlated with geographic distance for the whole sampling area, indicating together with the other analyses, that Emscher and Berne represent strong migration barriers and that passive dispersal probably only plays a role at a more local scale for this species. However, like in *A. aquaticus* genetic differentiation

640 was in some cases higher than expected based on distance alone. The similarities between
641 differentiation patterns in both species, even though they only co-occurred at four sites,
642 suggest that similar migration or gene flow barriers lead to the isolation of populations as
643 discussed for *A. aquaticus*.
644
645 **Conclusions**
646 Against our expectations, we found a strong small-scale population structure within both
647 species. This underlines the analytical power and importance of using high-resolution genetic
648 markers to analyze metapopulations and to identify potential barriers of migration or gene flow.
649 While we could identify some migration barriers and found indications for passive dispersal
650 probably by either birds or humans, further studies are needed to disentangle how effects of
651 isolation by dispersal limitations, local adaptation and biotic interactions shape metapopulation
652 structure. Our study shows that a high local macroinvertebrate population genetic diversity has
653 been maintained in the Emscher system, thus pointing at a high conservation value even of
654 urban streams. Finally, we could also show the importance of integrating nuclear markers into
655 species delimitation, especially in isopod species, where an extremely high COI diversity can
656 be found within species. Here, we showed that both, OTU A and J in *A. aquaticus*, and MOTU
657 240 and 241 in *P. coxalis*, represent only one species, each, in the sampling area. While final
658 assessment should include more regions, the sympatric occurrence of both COI groups in each
659 of the species suggests that they could generally be considered as one species in their
660 distribution range.

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Figure Legends

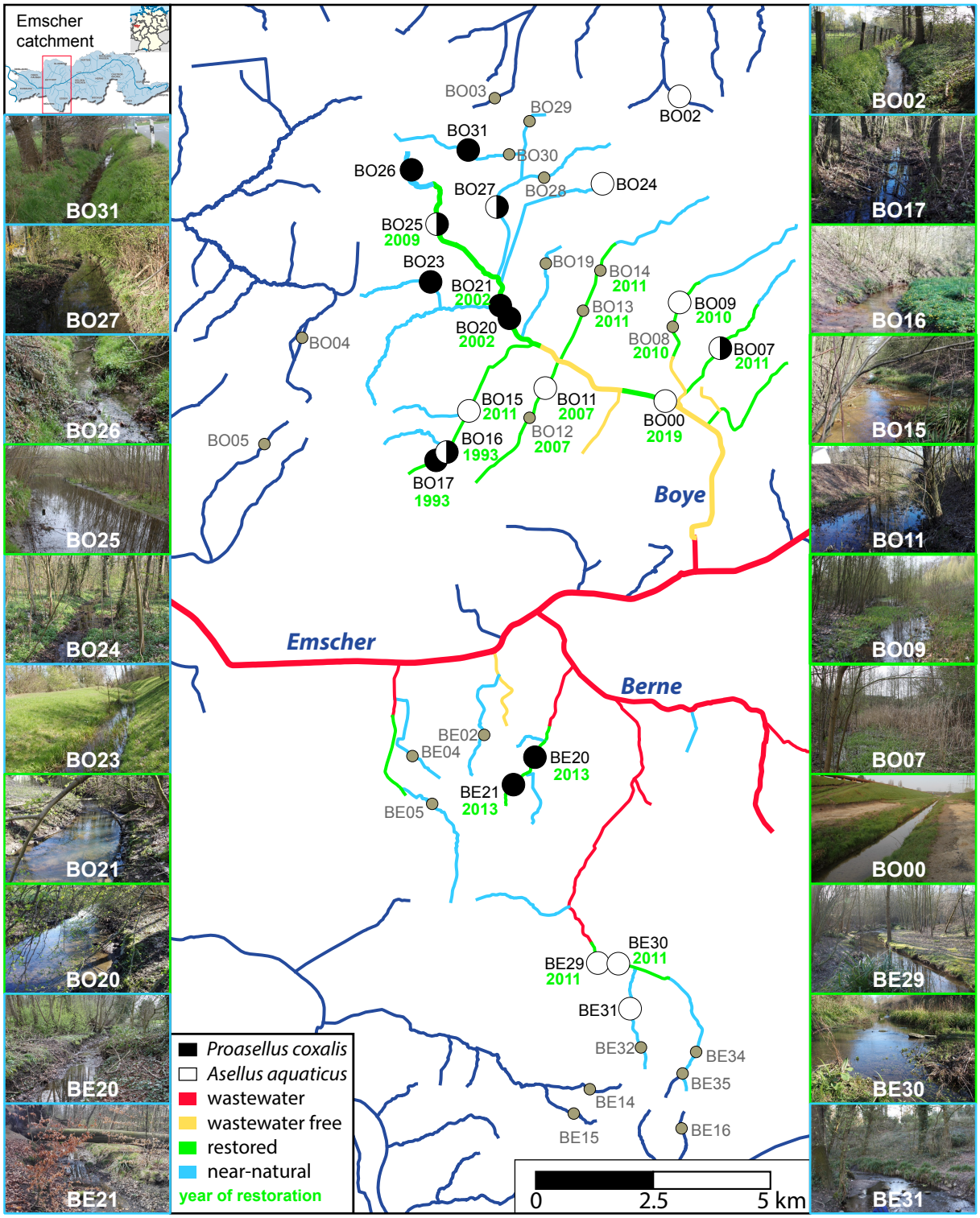

Fig. 1: Location of sampling sites within Boye and Berne catchment with indication where *A. aquaticus* and *P. coxalis* were found. Further, the ecological status in terms of restoration in 2019/2020 and the year of the restoration are shown below sampling sites, where the species were found. On the side, exemplary pictures (Lea Heberle) and the location of the sampling area*1 in Germany*2 are shown.

*1 (https://www.umwelt.nrw.de/system/files/media/images/2015-07/Karte.jpg); *2(https://en.m.wikipedia.org/wiki/File:Locator_map_RVR_in_Germany.svg)

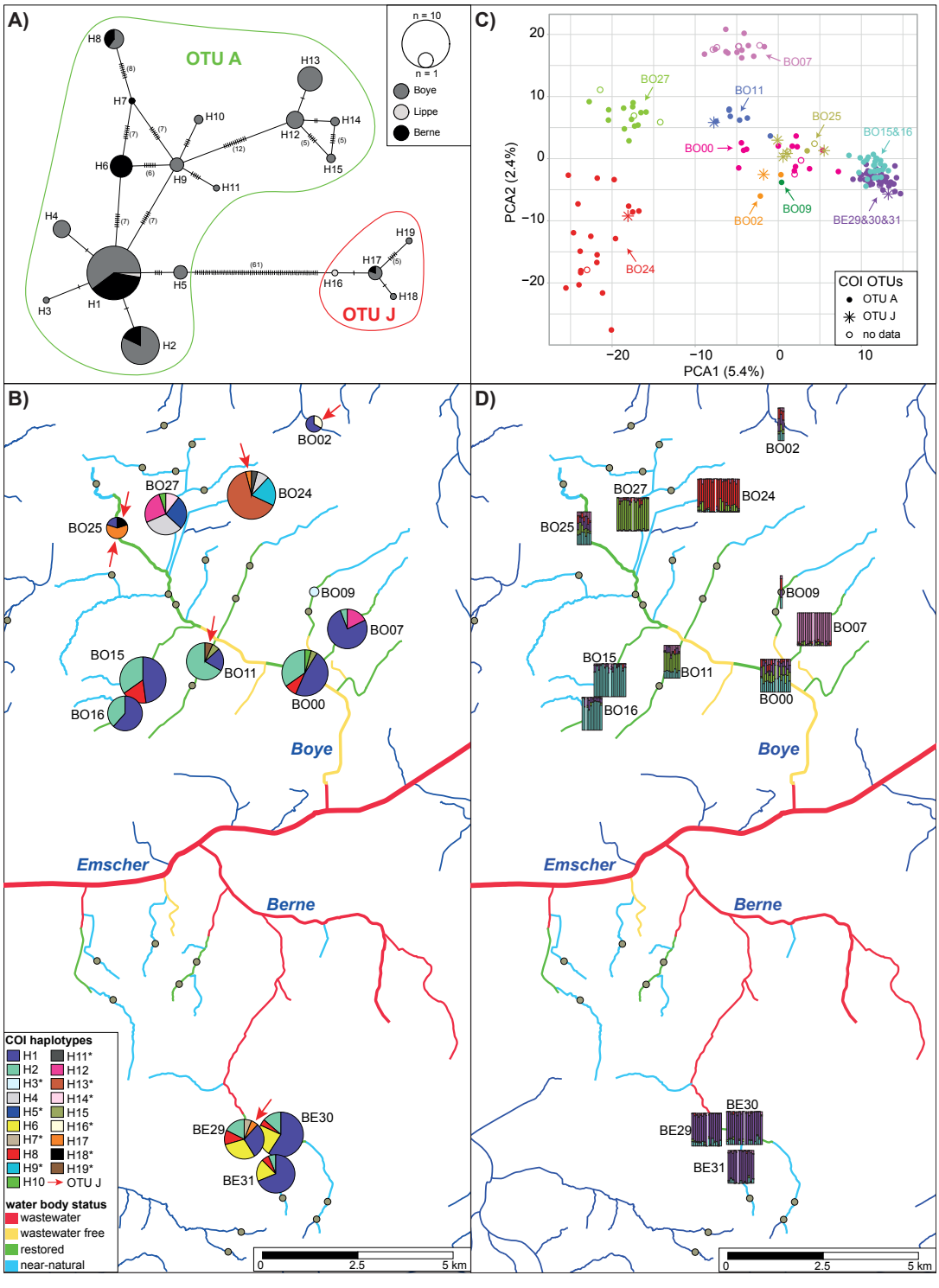

Fig. 2: Population structure of *A. aquaticus* in the Emscher catchment. A) COI minimum spanning network colored according to catchments. Vertical lines indicate mutations between haplotypes. B) COI haplotype map showing the haplotype composition. The sizes of haplotype pie charts are scaled according to the numbers of sequences per site and haplotypes of OTU J are indicated by an arrow. C) Principle component analysis of the ddRAD-seq data. Individuals are colored according to location and different symbols used for different COI OTUs. D) Ancestry estimates from sNMF analysis for k=5 displayed on the map, with vertical bars representing individual ancestry coefficients.

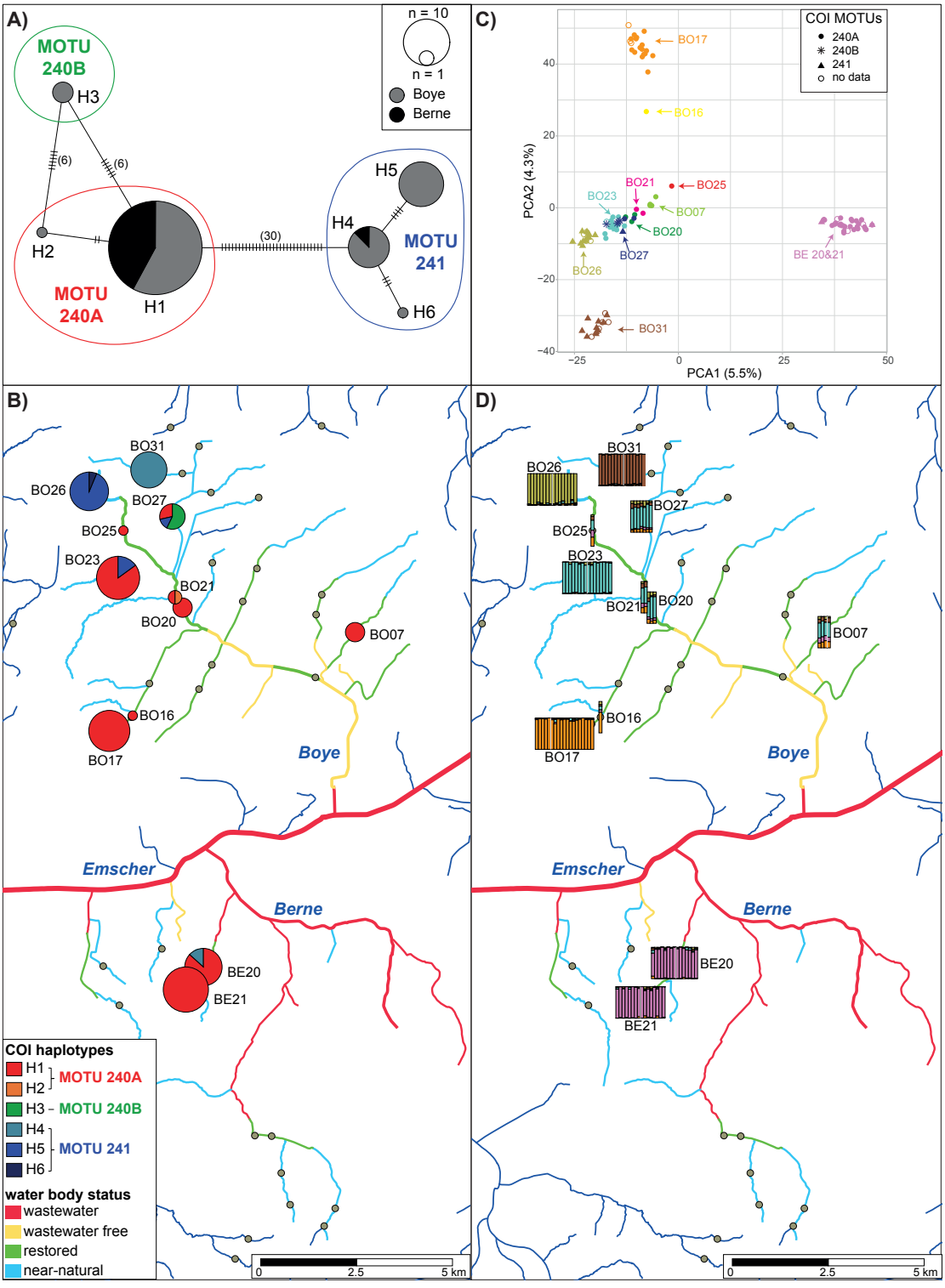

Fig. 3: Population structure of *P. coxalis* in the Emscher catchment. A) COI minimum spanning network colored according to catchments. Vertical lines indicate mutations between haplotypes. B) COI haplotype map showing the haplotype composition. The sizes of haplotype pie charts are scaled according to the numbers of sequences per site. C) Principle component analysis of the ddRAD-seq data. Individuals are colored according to location and different symbols used for different COI OTUs. D) Ancestry estimates from sNMF analysis for k=5 displayed on the map, with vertical bars representing individual ancestry coefficients.

Supplementary Table and Figure Legends

Tab. S1: Sampling sites with coordinates (WGS84), sampling dates for both years, stream name, catchment affiliation, and ecological state. Further, the number of specimens analyzed per genetic marker is given (in brackets) together with the number of specimens in the final analysis for each sampling site.

Tab. S2. Information for ddRAD library preparation per sample for *A. aquaticus* and *P. coxalis*, number of missing loci in final data set, indication if used in final analysis after filtering, COI haplotype and BOLD (COI data) and NCBI (ddRAD data) accession numbers. Below, also haplotype information on specimens is given, for which only COI sequences were generated.

Tab. S3: Haplotype distribution for *A. aquaticus*. Given are numbers for each year and for both years together (indicated with grey background).

Tab. S4: Results for the best partitions found by ASAP using K80 as a substitution model for *A. aquaticus* and *P. coxalis*.

Tab. S5: Different measures for genetic diversity for both species per sampling site. Diversity measures for ddRAD are: allelic richness (AR) and observed heterozygosity (H_O). Measures for COI are haplotype diversity (HDiv) and nucleotide diversity (NDiv). n is the number of specimens for each marker. Diversity was only calculated for sites with n > 5.)

Tab. S6: Summary statistics for all stacks settings for A. *aquaticus* and *P. coxalis* (Test) and for the final dataset (Final); loci limit = percentage of specimens required to have the loci, ma = minor allele frequency, H_O = observed heterozygosity, H_S = within population gene diversity, H_T = overall gene diversity, best K: K with lowest cross-entropy (median from all repetitions) in sNMF analysis.

Tab. S7: Haplotype distribution for *P. coxalis*. Given are numbers for each year and for both years together (indicated with grey background).

Fig. S1: F_{ST} heat maps for pairwise comparisons between sampling sites for *A. aquaticus* (A, B) and *P. coxalis* (C, D) and COI (A, C) and ddRAD data (B, D), respectively. Above the diagonal pairwise F_{ST} values are given and below either p-values (COI data sets; values < 0.05 indicate significant differentiation) or the lower confidence interval (ddRAD data set; values > 0 indicate significant differentiation) are given. In the diagonal, F_{ST} values for the comparison between the years 2019 and 2020 are given, with a white square, when only samples from one year were available. Significant F_{ST} values are indicated in bold and all values are colored according to the level of differentiation.

Fig. S2: Correlation between pairwise genetic distances (F_{ST}; A and B COI data, C and D ddRAD data) and waterway distances for *A. aquaticus* (A, C) and *P. coxalis* (B, D).

Fig. S3: Neighbor net of *A. aquaticus* for the ddRAD data set. Branches are colored according to sampling sites.

Fig. S4: PCA of the ddRAD data of A) *A. aquaticus* and D) *P. coxalis*, with the first plot showing the 1st and the 2nd axis and the second plot showing the 1st and the 3rd axis, respectively. B) and C) show standard boxplots of cross-entropy values (40 repeats) of sNMF analysis for final ddRAD datasets for *A. aquaticus* and *P. coxalis*, respectively.

Fig. S5: Relative migration networks (D) for A) *A. aquaticus* and B) *P. coxalis*. Only populations with >5 individuals were included in the analysis.

Fig. S6: Neighbor net of *P. coxalis* for the ddRAD data set. Branches are colored according to sampling sites.

Data Accessibility Statement

The data that support the findings of this study will be available at different NCBI BioProjects with the accession numbers XXXX for *A. aquaticus* and YYYYYY for *P. coxalis* upon publication. Additionally, demultiplexed ddRAD data used for Stacks clustering will be uploaded as BioSamples and individual accession numbers will be given in Table S2. COI haplotype sequences will be available under the NCBI-Accession numbers XXX – XXX, indicated in Table S2.

Competing Interests Statement

The authors have no conflicts of interest to declare.

Author Contributions

Martina Weiss: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Funding acquisition (supporting); Investigation (lead); Methodology (lead); Project administration (supporting); Visualization (lead); Writing – original draft (lead); Writing – review & editing (lead).
Armin W. Lorenz: Conceptualization (supporting); Investigation (supporting); Writing – review & editing (supporting).
Christian K. Feld: Investigation (supporting); Writing – review & editing (supporting).
Florian Leese: Conceptualization (equal); Data curation (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Writing – original draft (supporting); Writing – review & editing (supporting).
All authors have approved the final version of the manuscript.

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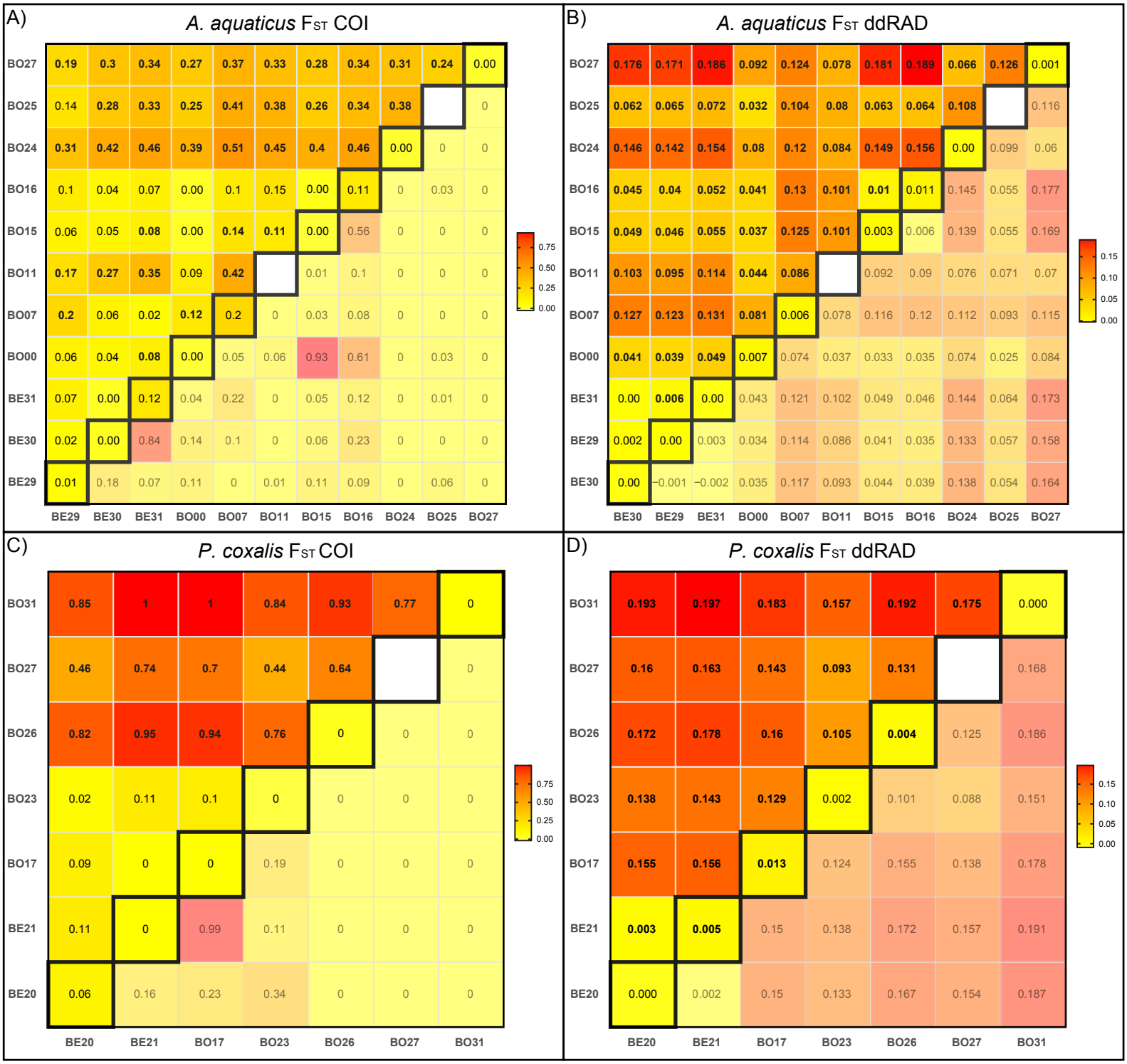

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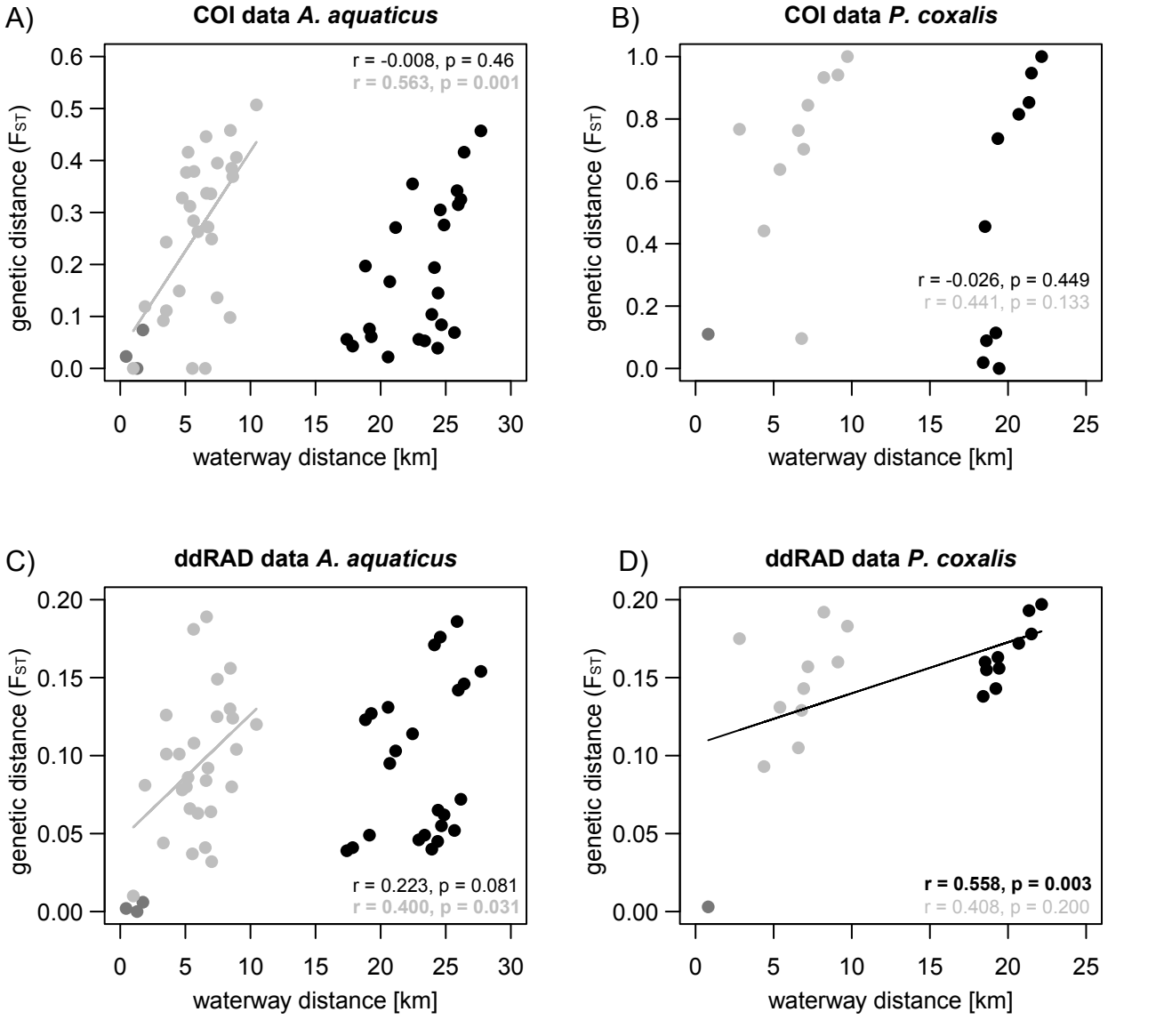

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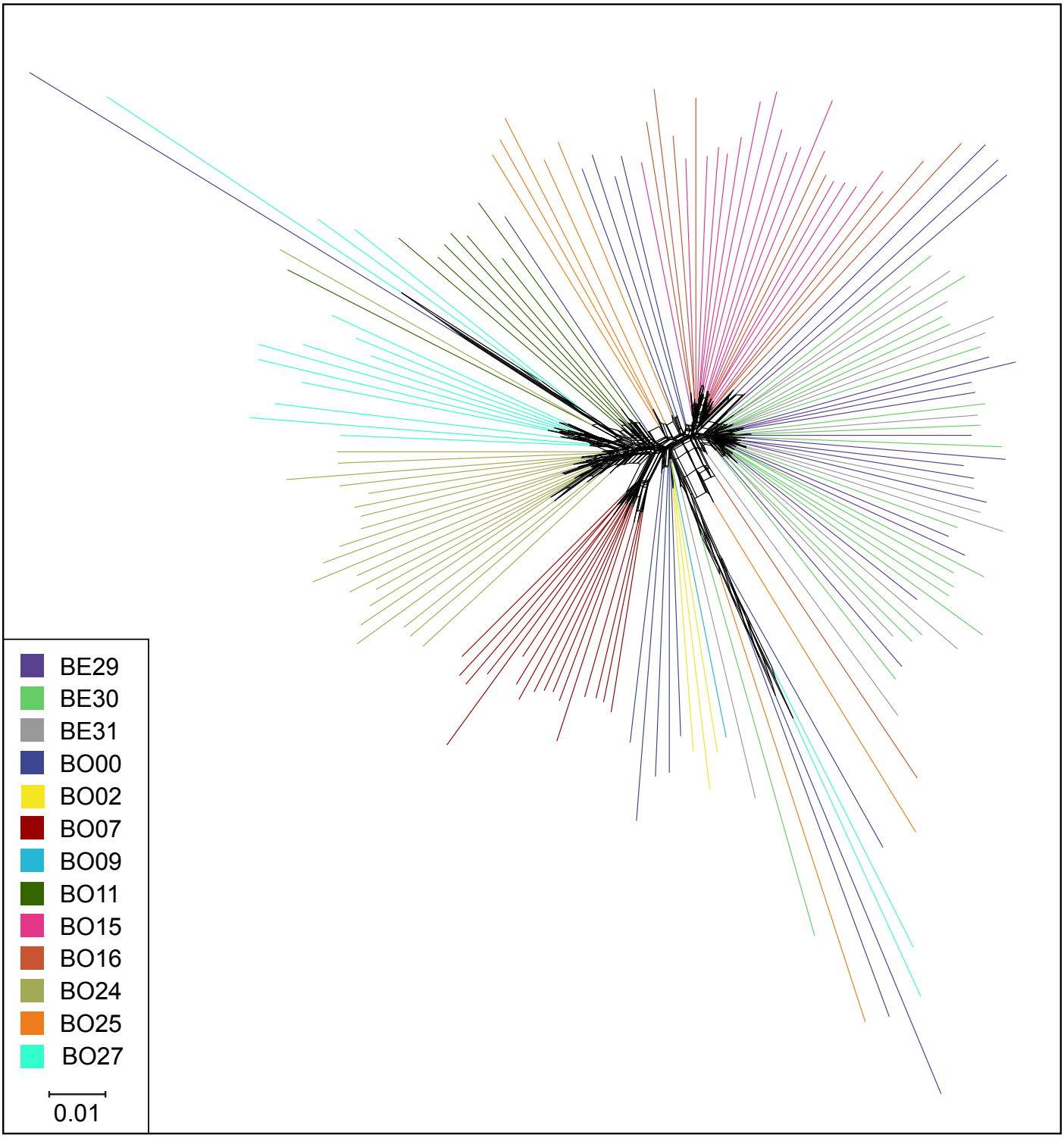

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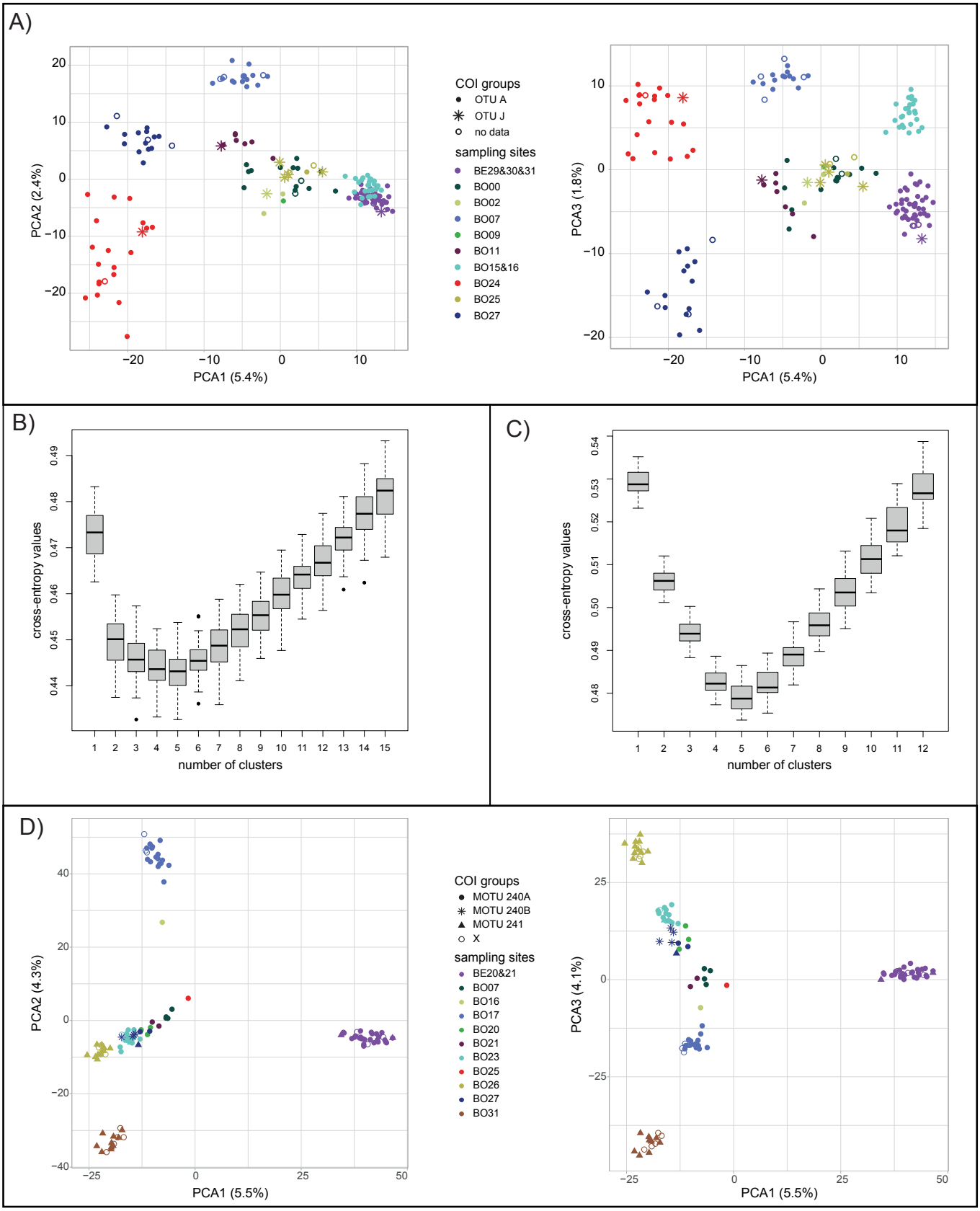

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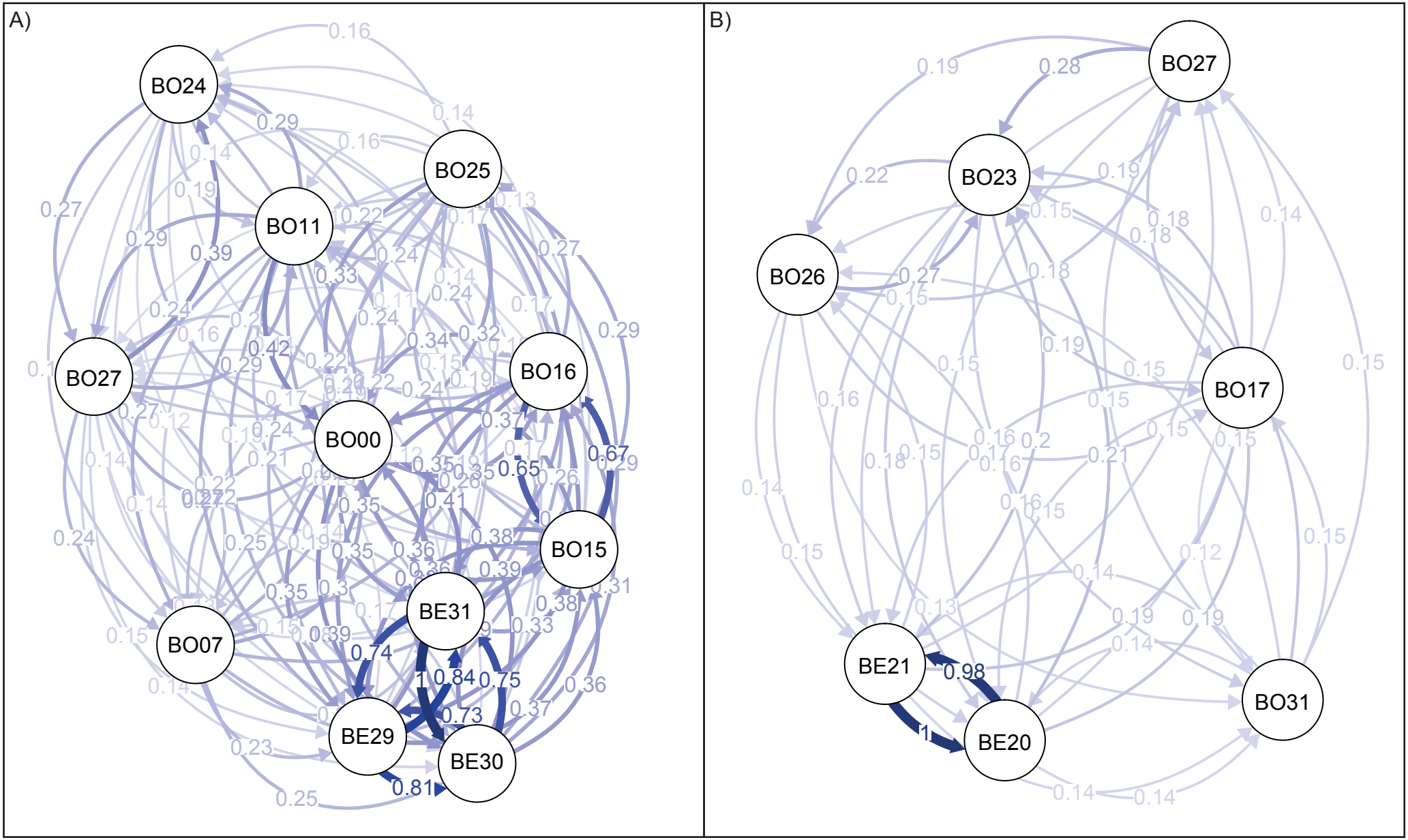

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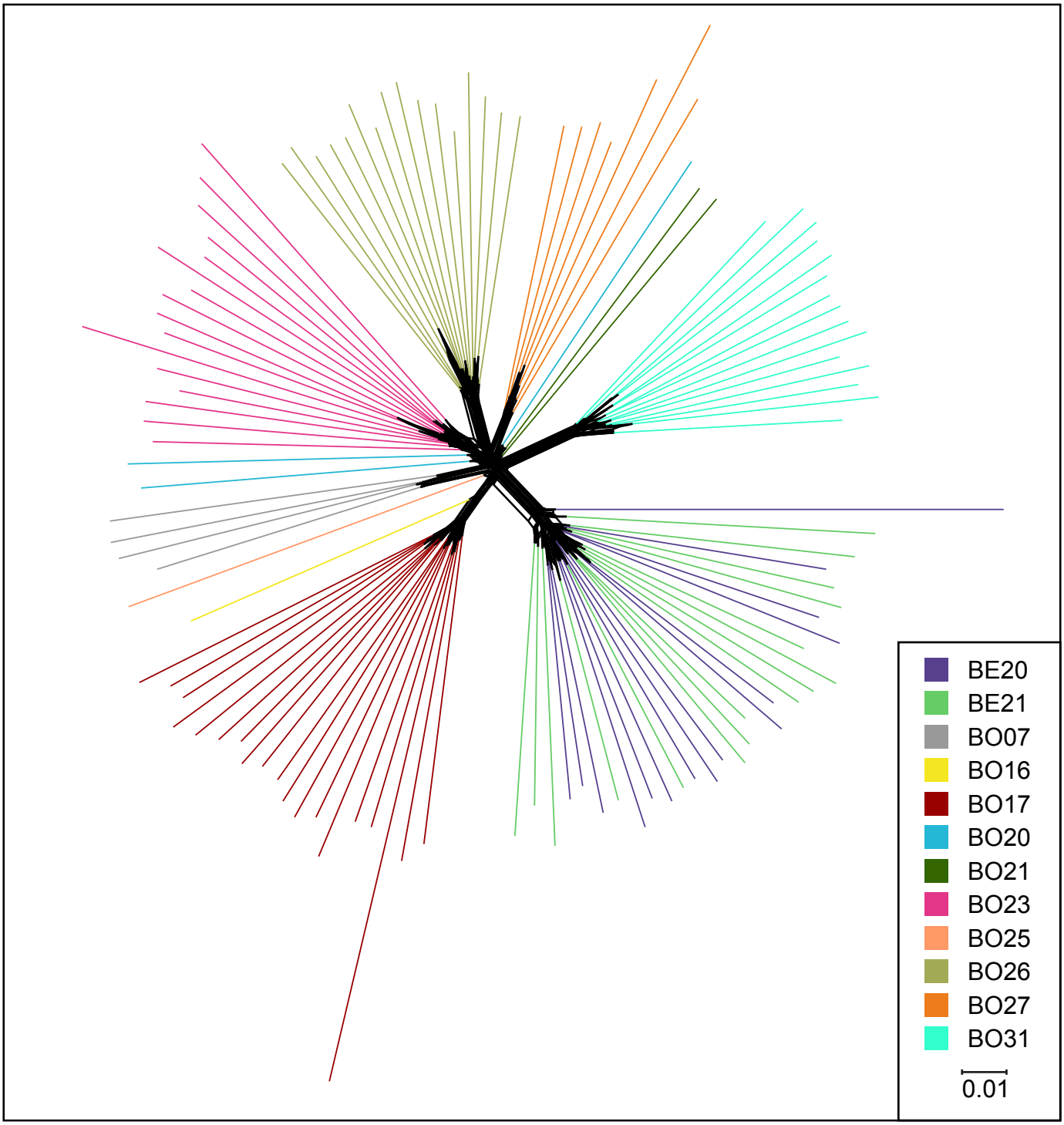

Fig. S6: Neighbor net of *P. coxalis* for the ddRAD data set. Branches are colored according to sampling sites.

Tab. S1: Sampling sites with coordinates (WGS84), sampling dates for both years, stream name, catchment affiliation, and ecological state. Further, the number of specimens analyzed per genetic marker is given (in brackets) together with the number of specimens in the final analysis for each sampling site.

Site	coordinates		Sampling date		Stream name	Catchment	Ecological state	*Proasellus coxalis*				*Asellus aquaticus*			
	Latitude	Longitude	2019	2020				COI 2019	ddRAD 2019	COI 2020	ddRAD 2020	COI 2019	ddRAD 2019	COI 2020	ddRAD 2020
BO00	51,544942	6,982729	05.04.19	21.04.20	Boye	Boye (Emscher)	restored 2019	0	0	0	0	8 (10)	8 (9)	15 (15)	7 (9)
BO02	51,603472	6,985075	08.04.19	16.04.20	Mühlenbach	Lippe	near-natural	0	0	0	0	0	0	3 (3)	3 (3)
BO07	51,555484	6,997598	02.04.19	21.04.20	Nattbach	Boye (Emscher)	restored 2011	3 (3)	3 (3)	1 (1)	1 (1)	4 (8)	8 (8)	13 (13)	9 (9)
BO09	51,564201	6,985123	02.04.19	21.04.20	Wittringer Mühlenbach	Boye (Emscher)	restored 2010	0	0	0	0	1 (1)	1 (1)	0	0
BO11	51,547875	6,943973	08.04.19	21.04.20	Kirchschemmsbach	Boye (Emscher)	restored 2007	0	0	0	0	0	0	15 (15)	8 (8)
BO15	51,543524	6,920588	29.03.19	22.04.20	Vorthbach	Boye (Emscher)	restored 2011	0	0	0	0	9 (9)	8 (8)	14 (14)	8 (8)
BO16	51,535679	6,913837	29.03.19	22.04.20	Vorthbach	Boye (Emscher)	restored 1993	0	0	1 (1)	1 (1)	2 (2)	2 (2)	11 (11)	8 (8)
BO17	51,534091	6,910535	29.03.19	22.04.20	Vorthbach	Boye (Emscher)	restored 1993	5 (6)	5 (5)	13 (15)	14 (14)	0	0	0	0
BO20	51,561190	6,932998	08.04.19	20.04.20	Boye	Boye (Emscher)	restored 2002	4 (4)	3 (3)	0	0	0	0	0	0
BO21	51,563604	6,930191	29.03.19	20.04.20	Boye	Boye (Emscher)	restored 2002	0	0	2 (2)	2 (2)	0	0	0	0
BO23	51,568118	6,908860	01.04.19	20.04.20	Schöttelbach	Boye (Emscher)	near-natural	8 (9)	8 (8)	12 (12)	8 (8)	0	0	0	0
BO24	51,586814	6,961502	01.04.19	20.04.20	Quaelingsbach	Boye (Emscher)	near-natural	0	0	0	0	9 (10)	9 (9)	16 (16)	12 (12)
BO25	51,579125	6,910851	08.04.19	20.04.20	Boye	Boye (Emscher)	restored 2009	0	0	1 (1)	1 (1)	0	0	5 (7)	7 (7)
BO26	51,589471	6,903014	04.04.19	16.04.20	Boye	Boye (Emscher)	near-natural	7 (9)	8 (8)	9 (12)	8 (8)	0	0	0	0
BO27	51,582391	6,929193	04.04.19	16.04.20	Brabecker Mühlenbach	Boye (Emscher)	near-natural	0	0	7 (7)	7 (7)	8 (10)	8 (9)	11 (12)	8 (8)
BO31	51,593251	6,920382	05.04.19	16.04.20	Wiesentalbach	Boye (Emscher)	near-natural	7 (8)	8 (8)	7 (12)	7 (8)	0	0	0	0
BE20	51,477440	6,940806	19.03.19	15.04.20	Pausmühlenbach	Berne (Emscher)	restored 2013	9 (10)	8 (8)	6 (7)	7 (7)	0	0	0	0
BE21	51,472198	6,934231	25.03.19	15.04.20	Pausmühlenbach	Berne (Emscher)	restored 2013	9 (10)	8 (8)	13 (13)	8 (8)	0	0	0	0
BE29	51,438118	6,960072	21.03.19	27.04.20	Borbecker Mühlenbach	Berne (Emscher)	restored 2011	0	0	0	0	10 (10)	8 (8)	7 (7)	7 (7)
BE30	51,437993	6,966351	21.03.19	27.04.20	Borbecker Mühlenbach	Berne (Emscher)	restored 2011	0	0	0	0	9 (10)	9 (9)	13 (14)	9 (9)
BE31	51,429386	6,969942	22.03.19	27.04.20	Kesselbach	Berne (Emscher)	near-natural	0	0	0	0	3 (5)	5 (5)	13 (13)	8 (8)
BO03	51,603125	6,928509	08.04.19	16.04.20	Schölsbach	Lippe	near-natural	0	0	0	0	0	0	0	0
BO04	51,557473	6,869515	08.04.19	22.04.20	Ebersbach	Rhine	near-natural	0	0	0	0	0	0	0	0
BO05	51,537199	6,857960	08.04.19	22.04.20	Rotbach	Rhine	near-natural	0	0	0	0	0	0	0	0
BO08	51,559560	6,983006	02.04.19	21.04.20	Wittringer Mühlenbach	Boye (Emscher)	restored 2010	0	0	0	0	0	0	0	0
BO12	51,542215	6,939166	29.03.19	21.04.20	Kirchschemmsbach	Boye (Emscher)	restored 2007	0	0	0	0	0	0	0	0
BO13	51,562628	6,955543	29.03.19	20.04.20	Haarbach	Boye (Emscher)	restored 2011	0	0	0	0	0	0	0	0
BO14	51,570292	6,960856	29.03.19	20.04.20	Haarbach	Boye (Emscher)	restored 2011	0	0	0	0	0	0	0	0
BO19	51,571629	6,944156	04.04.19	20.04.20	Alter Haarbach	Boye (Emscher)	near-natural	0	0	0	0	0	0	0	0
BO28	51,587987	6,943634	01.04.19	16.04.20	Brabecker Mühlenbach	Boye (Emscher)	near-natural	0	0	0	0	0	0	0	0
BO29	51,598736	6,939163	08.04.19	16.04.20	Bornemannsbach	Boye (Emscher)	near-natural	0	0	0	0	0	0	0	0
BO30	51,592411	6,932909	05.04.19	16.04.20	Wiesentalbach	Boye (Emscher)	near-natural	0	0	0	0	0	0	0	0
BE02	51,481699	6,925260	20.03.19	15.04.20	Barchembach	Emscher	near-natural	0	0	0	0	0	0	0	0
BE04	51,477657	6,903294	25.03.19	15.04.20	Heilgraben	Emscher	near-natural	0	0	0	0	0	0	0	0
BE05	51,468529	6,909343	25.03.19	15.04.20	Hexbach	Emscher	near-natural	0	0	0	0	0	0	0	0
BE14	51,413991	6,957609	28.03.19	15.04.20	Steinbach	Ruhr	near-natural	0	0	0	0	0	0	0	0
BE15	51,409337	6,952697	26.03.19	15.04.20	Ruhmbach	Ruhr	near-natural	0	0	0	0	0	0	0	0
BE16	51,406501	6,985709	26.03.19	27.04.20	Wolfsbach	Ruhr	near-natural	0	0	0	0	0	0	0	0
BE32	51,421979	6,973276	22.03.19	27.04.20	Kesselbach	Berne (Emscher)	near-natural	0	0	0	0	0	0	0	0
BE34	51,421128	6,990172	22.03.19	27.04.20	Borbecker Mühlenbach	Berne (Emscher)	near-natural	0	0	0	0	0	0	0	0
BE35	51,417001	6,986024	22.03.19	27.04.20	Borbecker Mühlenbach	Berne (Emscher)	near-natural	0	0	0	0	0	0	0	0
								52 (59)	**51 (51)**	**72 (83)**	**64 (65)**	**63 (75)**	**66 (68)**	**136 (140)**	**94 (96)**

Tab S2. Information for ddRAD library preparation per sample for *A. aquaticus* and *P. coxalis* , number of missing loci in final data set, indication if used in final analysis after filtering, COI haplotype and BOLD (COI data) and NCBI (ddRAD data) accession numbers. Below, also haplotype information on specimens is given, for which only COI sequences were generated.

species	ID	sample name	initial DNA concentration [ng/µl]	used amount of DNA [ng]	name P5 adapter	volume P5 adapter [µl]	name P7 adapter	volume P7 adapter [µl]	final DNA concentration in target size range (1. PCR) [ng/µl]	final total DNA concentration (1. PCR) [ng/µl]	final DNA concentration in target size range (2. PCR) [ng/µl]	final total DNA concentration (2. PCR) [ng/µl]	library number	amount of target sized DNA in library [ng]	total amount of DNA in library [ng]	number of missing loci [%]	used in final ddRAD analysis?	COI haplotype	BOLD accession number	NCBI Biosample accession number
A. aquaticus	AA1	AA_BO27_190401_04	68,0	600	1	1,2	11	3,3	4,31	6,87			1	12,6	20	0,096	yes	H12		
A. aquaticus	AA2	AA_BO00_190402_02	51,6	600	2	1,2	11	3,3	10,39	13,89			1	15,0	20	0,088	yes	H1		
A. aquaticus	AA3	AA_BO27_190401_01	48,0	600	3	1,2	11	3,3	2,17	2,72			1	15,9	20	0,055	yes	only species determination		
A. aquaticus	AA4	AA_BO27_190401_02	45,8	600	4	1,2	11	3,3	2,78	4,46			1	12,5	20	0,204	yes	H5		
A. aquaticus	AA5	AA_BO27_190401_10	45,8	600	5	1,2	11	3,3	9,90	13,89			1	14,3	20	0,082	yes	H12		
A. aquaticus	AA6	AA_BO27_190401_03	44,4	600	6	1,2	11	3,3	7,01	11,97			1	11,7	20	0,186	yes	H5		
A. aquaticus	AA7	AA_BO27_190401_08	44,2	600	7	1,2	11	3,3	9,34	12,63			1	14,8	20	0,066	yes	H4		
A. aquaticus	AA8	AA_BO00_190402_06	40,2	600	8	1,2	11	3,3	4,67	8,15			1	11,5	20	0,121	yes	H2		
A. aquaticus	AA09	AA_BO27_190401_09	39,2	600	9	1,2	11	3,3	2,16	3,12			1	13,8	20	0,241	yes	only species determination		
A. aquaticus	AA10	AA_BO27_190401_07	39,0	600	10	1,2	11	3,3	2,27	2,73			1	16,7	20	0,071	yes	H5		
A. aquaticus	AA11	AA_BO00_190402_05	37,4	600	11	1,2	11	3,3	0,25	0,32	0,661	1,0686	1	12,4	20	0,419	no	H2		
A. aquaticus	AA12	AA_BE31_190322_01	36,0	600	12	1,2	11	3,3	1,61	2,50			1	12,9	20	0,071	yes	H8		
A. aquaticus	AA13	AA_BO00_190402_01	34,8	600	13	1,2	11	3,3	2,18	2,86			1	15,2	20	0,103	yes	only species determination		
A. aquaticus	AA14	AA_BO00_190402_03	34,8	600	14	1,2	11	3,3	1,95	4,41			1	8,8	20	0,289	yes	only species determination		
A. aquaticus	AA15	AA_BO27_200416_15	33,9	600	15	1,2	11	3,3	2,67	3,30			1	16,2	20	0,097	yes	H4		
A. aquaticus	AA16	AA_BO27_190401_06	33,2	600	16	1,2	11	3,3	1,90	2,64			1	14,4	20	0,84	no	H4		
A. aquaticus	AA17	AA_BO24_200420_11	33,2	600	18	1,2	11	3,3	0,29	0,68	3,9294	5,3132	1	14,8	20	0,045	yes	H9		
A. aquaticus	AA18	AA_BO25_200420_06	33,1	600	19	1,2	11	3,3	7,54	8,71			1	17,3	20	0,037	yes	H17		
A. aquaticus	AA19	AA_BO24_190408_03	32,8	600	20	1,2	11	3,3	1,28	1,84			1	13,9	20	0,105	yes	H13		
A. aquaticus	AA20	AA_BO00_200421_14	32,5	600	21	1,2	11	3,3	0,70	1,27			1	11,0	20	0,54	no	H1		
A. aquaticus	AA21	AA_BO00_190402_07	32,2	600	22	1,2	11	3,3	1,83	3,67			1	10,0	20	0,224	yes	H15		
A. aquaticus	AA22	AA_BO00_190402_04	31,6	600	23	1,2	11	3,3	4,36	5,68			1	15,4	20	0,029	yes	H1		
A. aquaticus	AA23	AA_BO15_190329_01	30,0	600	25	1,2	11	3,3	4,27	5,45			1	15,7	20	0,038	yes	H2		
A. aquaticus	AA24	AA_BO27_200416_12	29,5	600	27	1,2	11	3,3	6,91	8,92			1	15,5	20	0,151	yes	H5		
A. aquaticus	AA25	AA_BO27_200416_14	29,3	600	1	1,2	8	3,3	3,71	4,65			1	16,0	20	0,094	yes	H5		
A. aquaticus	AA26	AA_BE30_190321_03	29,0	600	2	1,2	8	3,3	2,80	3,20			1	17,5	20	0,034	yes	H1		
A. aquaticus	AA27	AA_BO27_200416_11	27,9	600	3	1,2	8	3,3	7,74	9,12			1	17,0	20	0,035	yes	H10		
A. aquaticus	AA28	AA_BO00_190402_08	26,8	600	4	1,2	8	3,3	5,89	6,91			1	17,1	20	0,023	yes	H1		
A. aquaticus	AA29	AA_BO24_200420_15	26,4	600	5	1,2	8	3,3	0,67	1,23	4,6475	6,2726	1	14,8	20	0,03	yes	H13		
A. aquaticus	AA30	AA_BO24_200420_19	26,3	600	6	1,2	8	3,3	0,22	0,59	3,9515	5,0563	1	15,6	20	0,029	yes	H13		
A. aquaticus	AA31	AA_BO00_200421_18	26,1	600	7	1,2	8	3,3	0,48	1,11	4,6215	6,4706	1	14,3	20	0,061	yes	H2		
A. aquaticus	AA32	AA_BO24_190408_05	26,0	600	8	1,2	8	3,3	6,05	7,35			1	16,5	20	0,035	yes	H13		
A. aquaticus	AA33	AA_BO16_190329_01	25,8	600	9	1,2	8	3,3	6,26	7,75			1	16,1	20	0,035	yes	H1		
A. aquaticus	AA34	AA_BO24_200420_13	25,4	600	10	1,2	8	3,3	5,12	6,41			1	16,0	20	0,049	yes	H9		
A. aquaticus	AA35	AA_BO16_200422_08	25,3	600	11	1,2	8	3,3	3,64	4,59			1	15,9	20	0,101	yes	H1		
A. aquaticus	AA36	AA_BO16_200422_06	25,0	600	12	1,2	8	3,3	6,49	7,91			1	16,4	20	0,046	yes	H1		
A. aquaticus	AA37	AA_BO24_200420_20	24,5	613	13	1,2	8	3,3	9,40	11,51			1	16,3	20	0,044	yes	H13		
A. aquaticus	AA38	AA_BO24_190408_02	24,2	605	14	1,2	8	3,3	2,72	3,47			1	15,7	20	0,08	yes	H13		
A. aquaticus	AA39	AA_BO15_200422_24	24,2	605	15	1,2	8	3,3	7,16	8,33			1	17,2	20	0,037	yes	H1		
A. aquaticus	AA40	AA_BE31_200427_14	23,8	595	16	1,2	8	3,3	3,75	4,34			1	17,3	20	0,084	yes	H1		
A. aquaticus	AA41	AA_BO00_190402_10	23,4	500	18	1	8	2,8	0,84	1,01			1	16,5	20	0,049	yes	H8		
A. aquaticus	AA42	AA_BO00_200421_12	23,4	500	19	1	8	2,8	1,11	1,91			1	11,6	20	0,41	no	H2		
A. aquaticus	AA43	AA_BO24_200420_12	23,3	500	20	1	8	2,8	4,76	6,21			1	15,3	20	0,061	yes	H4		
A. aquaticus	AA44	AA_BE29_190321_03	23,2	500	21	1	8	2,8	1,92	2,52			1	15,2	20	0,104	yes	H6		
A. aquaticus	AA45	AA_BO16_200422_07	22,6	500	22	1	8	2,8	1,43	2,18			1	13,1	20	0,092	yes	H2		
A. aquaticus	AA46	AA_BO24_190408_04	22,4	500	23	1	8	2,8	6,81	7,95			1	17,1	20	0,035	yes	H9		
A. aquaticus	AA47	AA_BO24_190408_09	22,4	500	25	1	8	2,8	7,01	8,38			1	16,7	20	0,028	yes	only species determination		
A. aquaticus	AA48	AA_BO25_200420_02	22,3	500	27	1	8	2,8	2,33	2,91			1	16,0	20	0,067	yes	H17		
A. aquaticus	AA49	AA_BO25_200420_04	22,3	500	1	1	10	2,8	2,42	3,95			1	12,2	20	0,214	yes	H1		
A. aquaticus	AA50	AA_BO16_200422_05	22,1	500	2	1	10	2,8	6,88	9,04			1	15,2	20	0,064	yes	H1		
A. aquaticus	AA51	AA_BO24_190408_06	21,8	500	3	1	10	2,8	9,37	11,38			1	16,5	20	0,031	yes	H13		
A. aquaticus	AA52	AA_BO16_200422_09	21,8	500	4	1	10	2,8	5,55	6,45			1	17,2	20	0,035	yes	H2		
A. aquaticus	AA53	AA_BE29_190321_05	21,6	500	5	1	10	2,8	0,60	0,75	2,0357	2,7744	1	14,7	20	0,055	yes	H1		
A. aquaticus	AA54	AA_BO24_200420_22	21,5	500	6	1	10	2,8	4,78	5,77			1	16,6	20	0,028	yes	H13		
A. aquaticus	AA55	AA_BO27_200416_13	21,5	500	7	1	10	2,8	1,46	1,96			1	14,8	20	0,081	yes	H14		
A. aquaticus	AA56	AA_BE30_190321_05	21,4	500	8	1	10	2,8	0,50	0,75	0,8041	1,2726	1	12,6	20	0,17	yes	H6		
A. aquaticus	AA57	AA_BE30_190321_02	20,6	515	9	1	10	2,8	2,70	3,60			1	15,0	20	0,052	yes	H1		
A. aquaticus	AA58	AA_BO24_190408_07	20,6	515	10	1	10	2,8	4,27	5,18			1	16,5	20	0,034	yes	H13		
A. aquaticus	AA59	AA_BO15_190329_03	20,4	510	11	1	10	2,8	6,86	8,39			1	16,4	20	0,028	yes	H1		
A. aquaticus	AA60	AA_BO24_190408_01	20,4	510	12	1	10	2,8	7,29	9,01			1	16,2	20	0,019	yes	H11		

A. aquaticus	AA61	AA_BO02_200416_03	19,6	400	13	0,8	10	2,2	4,93	5,81			1	17,0	20	0,038	yes	H16
A. aquaticus	AA62	AA_BO24_200420_18	19,4	400	14	0,8	10	2,2	2,90	3,44			1	16,9	20	0,043	yes	H4
A. aquaticus	AA63	AA_BO07_200421_15	19,3	400	15	0,8	10	2,2	6,10	6,97			1	17,5	20	0,046	yes	H1
A. aquaticus	AA64	AA_BE31_200427_09	19,1	400	16	0,8	10	2,2	5,86	8,16			1	14,3	20	0,038	yes	H1
A. aquaticus	AA65	AA_BO16_200422_04	18,7	400	18	0,8	10	2,2	2,24	2,67			1	16,7	20	0,057	yes	H1
A. aquaticus	AA66	AA_BO25_200420_07	18,4	400	19	0,8	10	2,2	2,28	3,81			1	12,0	20	0,12	yes	only species determination
A. aquaticus	AA67	AA_BE31_190322_02	18,0	400	20	0,8	10	2,2	5,25	6,47			1	16,2	20	0,04	yes	only species determination
A. aquaticus	AA68	AA_BO27_200416_16	17,9	400	21	0,8	10	2,2	6,42	8,46			1	15,2	20	0,035	yes	H4
A. aquaticus	AA69	AA_BO00_200421_11	17,8	400	22	0,8	10	2,2	5,10	6,14			1	16,6	20	0,035	yes	H10
A. aquaticus	AA70	AA_BO25_200420_03	17,7	400	23	0,8	10	2,2	6,22	8,09			1	15,4	20	0,063	yes	only species determination
A. aquaticus	AA71	AA_BE30_200427_17	17,6	400	25	0,8	10	2,2	6,00	7,17			1	16,7	20	0,041	yes	H1
A. aquaticus	AA72	AA_BE31_200427_07	17,6	400	27	0,8	10	2,2	6,17	8,46			1	14,6	20	0,05	yes	H6
A. aquaticus	AA73	AA_BO25_200420_01	17,4	400	1	0,8	1	2,2	2,98	3,54			1	16,9	20	0,068	yes	H17
A. aquaticus	AA74	AA_BO24_200420_21	17,3	400	2	0,8	1	2,2	4,59	5,43			1	16,9	20	0,025	yes	H13
A. aquaticus	AA75	AA_BE29_190321_07	17,2	400	3	0,8	1	2,2	4,64	5,98			1	15,5	20	0,03	yes	H1
A. aquaticus	AA76	AA_BO00_200421_17	16,9	400	4	0,8	1	2,2	3,84	4,69			1	16,4	20	0,052	yes	H2
A. aquaticus	AA77	AA_BO16_190329_02	16,8	400	5	0,8	1	2,2	2,48	3,21			1	15,5	20	0,047	yes	H1
A. aquaticus	AA78	AA_BE30_190321_08	16,6	415	6	0,8	1	2,2	1,86	2,57			1	14,5	20	0,048	yes	H1
A. aquaticus	AA79	AA_BO24_190408_08	16,6	415	7	0,8	1	2,2	4,66	5,58			1	16,7	20	0,025	yes	H9
A. aquaticus	AA80	AA_BO24_200420_26	16,6	415	8	0,8	1	2,2	5,70	6,74			1	16,9	20	0,033	yes	H9
A. aquaticus	AA81	AA_BO11_200421_02	16,4	410	9	0,8	1	2,2	5,73	6,94			1	16,5	20	0,023	yes	H2
A. aquaticus	AA82	AA_BO27_200416_17	16,2	405	10	0,8	1	2,2	5,79	6,85			1	16,9	20	0,032	yes	only species determination
A. aquaticus	AA83	AA_BE30_200427_15	16,2	405	11	0,8	1	2,2	3,87	4,65			1	16,6	20	0,033	yes	H2
A. aquaticus	AA84	AA_BE31_190322_03	16,0	400	12	0,8	1	2,2	2,14	2,55			1	16,8	20	0,043	yes	H6
A. aquaticus	AA85	AA_BO00_200421_13	16,0	400	13	0,8	1	2,2	3,99	4,89			1	16,3	20	0,029	yes	H1
A. aquaticus	AA86	AA_BE30_200427_12	16,0	400	14	0,8	1	2,2	4,55	5,62			1	16,2	20	0,022	yes	H1
A. aquaticus	AA87	AA_BE31_200427_12	16,0	400	15	0,8	1	2,2	4,63	5,77			1	16,1	20	0,036	yes	H1
A. aquaticus	AA88	AA_BO24_200420_14	15,9	398	16	0,8	1	2,2	5,47	7,31			1	15,0	20	0,053	yes	H13
A. aquaticus	AA89	AA_BO16_200422_10	15,7	393	18	0,8	1	2,2	5,15	6,35			1	16,2	20	0,04	yes	H1
A. aquaticus	AA90	AA_BE29_190321_01	15,5	388	19	0,8	1	2,2	5,56	6,94			1	16,0	20	0,028	yes	H2
A. aquaticus	AA91	AA_BO11_200421_06	15,5	388	20	0,8	1	2,2	5,30	6,46			1	16,4	20	0,036	yes	H1
A. aquaticus	AA92	AA_BO07_200421_12	15,1	378	21	0,8	1	2,2	6,73	8,21			1	16,4	20	0,025	yes	H1
A. aquaticus	AA93	AA_BO07_200421_13	15,1	378	22	0,8	1	2,2	3,01	3,65			1	16,5	20	0,034	yes	H1
A. aquaticus	AA94	AA_BE30_200427_14	15,1	378	23	0,8	1	2,2	4,64	5,50			1	16,9	20	0,037	yes	H6
A. aquaticus	AA95	AA_BO02_200416_01	15,0	375	25	0,8	1	2,2	2,07	2,52			1	16,4	20	0,06	yes	H1
A. aquaticus	AA96	AA_BO11_200421_04	15,0	375	27	0,8	1	2,2	4,21	5,18			1	16,3	20	0,102	yes	H15
A. aquaticus	AA97	AA_BE31_200427_11	15,0	500	1	1	11	2,8	8,72	10,39			2	33,6	40	0,062	yes	H1
A. aquaticus	AA98	AA_BO11_200421_05	14,6	500	2	1	11	2,8	8,91	11,02			2	32,3	40	0,054	yes	H2
A. aquaticus	AA99	AA_BO27_200416_18	14,5	500	3	1	11	2,8	6,35	7,80			2	32,6	40	0,055	yes	H4
A. aquaticus	AA100	AA_BE30_200427_11	14,5	500	4	1	11	2,8	7,58	9,78			2	31,0	40	0,04	yes	only species determination
A. aquaticus	AA101	AA_BO24_200420_25	14,3	500	5	1	11	2,8	6,43	8,18			2	31,4	40	0,038	yes	H17
A. aquaticus	AA102	AA_BO11_200421_03	14,2	500	6	1	11	2,8	7,84	9,78			2	32,1	40	0,035	yes	H2
A. aquaticus	AA103	AA_BO15_200422_16	13,9	500	7	1	11	2,8	8,19	10,27			2	31,9	40	0,048	yes	H1
A. aquaticus	AA104	AA_BO11_200421_09	13,7	500	8	1	11	2,8	10,19	13,34			2	30,6	40	0,046	yes	H2
A. aquaticus	AA105	AA_BO25_200420_05	13,6	500	9	1	11	2,8	7,77	9,35			2	33,2	40	0,043	yes	H18
A. aquaticus	AA106	AA_BO00_200421_20	13,5	500	10	1	11	2,8	7,05	9,61			2	29,3	40	0,041	yes	H1
A. aquaticus	AA107	AA_BE30_200427_13	13,5	500	11	1	11	2,8	7,26	9,01			2	32,2	40	0,037	yes	H1
A. aquaticus	AA108	AA_BO11_200421_08	13,4	500	12	1	11	2,8	9,11	12,22			2	29,8	40	0,039	yes	H2
A. aquaticus	AA109	AA_BO16_200422_03	13,4	500	13	1	11	2,8	0,02	0,05	3,0471	3,7125	2	32,8	40	0,062	yes	H1
A. aquaticus	AA110	AA_BO07_200421_25	13,3	500	14	1	11	2,8	9,02	10,68			2	33,8	40	0,034	yes	H1
A. aquaticus	AA111	AA_BO00_200421_16	13,2	500	15	1	11	2,8	1,39	1,85			2	30,0	40	0,307	yes	H1
A. aquaticus	AA112	AA_BO07_200421_19	13,0	500	16	1	11	2,8	8,48	10,58			2	32,1	40	0,032	yes	H2
A. aquaticus	AA113	AA_BE31_200427_06	12,5	500	18	1	11	2,8	8,90	11,62			2	30,6	40	0,037	yes	H2
A. aquaticus	AA114	AA_BO07_200421_24	12,0	400	19	0,8	11	2,2	7,99	9,91			2	32,3	40	0,019	yes	H12
A. aquaticus	AA115	AA_BE30_200427_18	11,4	400	20	0,8	11	2,2	9,62	13,39			2	28,7	40	0,036	yes	H6
A. aquaticus	AA116	AA_BO11_200421_07	11,2	358	21	0,8	11	2,2	8,62	11,47			2	30,1	40	0,031	yes	H19
A. aquaticus	AA117	AA_BO02_200416_02	11,0	400	22	0,8	11	2,2	6,28	7,94			2	31,6	40	0,042	yes	H1
A. aquaticus	AA118	AA_BE30_200427_16	10,9	400	23	0,8	11	2,2	9,11	11,91			2	30,6	40	0,03	yes	H6
A. aquaticus	AA119	AA_BE31_200427_15	10,8	400	25	0,8	11	2,2	8,02	10,93			2	29,3	40	0,035	yes	H1
A. aquaticus	AA120	AA_BO07_190402_01	14,3	400	27	0,8	11	2,2	6,39	7,99			2	32,0	40	0,022	yes	H1
A. aquaticus	AA121	AA_BE29_200427_11	9,8	400	1	0,8	8	2,2	2,97	3,85			2	30,9	40	0,099	yes	H2
A. aquaticus	AA122	AA_BO15_200422_22	9,7	400	2	0,8	8	2,2	7,93	9,57			2	33,2	40	0,037	yes	H1
A. aquaticus	AA123	AA_BE30_190321_07	13,7	370	3	0,8	8	2,2	8,89	11,11			2	32,0	40	0,033	yes	H2
A. aquaticus	AA124	AA_BE30_190321_09	13,5	374	4	0,8	8	2,2	3,93	5,80			2	27,1	40	0,054	yes	H1
A. aquaticus	AA125	AA_BO07_200421_18	9,0	350	5	0,7	8	1,9	7,92	10,19			2	31,1	40	0,02	yes	H1
A. aquaticus	AA126	AA_BO15_190329_02	12,7	350	6	0,7	8	1,9	8,33	10,26			2	32,5	40	0,03	yes	H2

A. aquaticus	AA127	AA_BO15_200422_23	8,8	350	7	0,7	8	1,9	6,24	7,07			2	35,3	40	0,061	yes	H2
A. aquaticus	AA128	AA_BO07_200421_20	8,6	350	8	0,7	8	1,9	10,21	12,81			2	31,9	40	0,035	yes	H1
A. aquaticus	AA129	AA_BO09_190402_01	12,1	300	9	0,6	8	1,7	8,76	11,92			2	29,4	40	0,039	yes	H3
A. aquaticus	AA130	AA_BO07_200421_16	8,4	300	10	0,6	8	1,7	10,55	14,07			2	30,0	40	0,031	yes	H1
A. aquaticus	AA131	AA_BO00_200421_19	8,3	300	11	0,6	8	1,7	10,76	13,80			2	31,2	40	0,035	yes	H8
A. aquaticus	AA132	AA_BE31_190322_04	11,8	300	12	0,6	8	1,7	10,08	13,18			2	30,6	40	0,026	yes	H1
A. aquaticus	AA133	AA_BO15_190329_08	11,7	300	13	0,6	8	1,7	0,38	0,69	3,7962	4,6319	2	32,8	40	0,042	yes	H1
A. aquaticus	AA134	AA_BE30_190321_01	11,6	300	14	0,6	8	1,7	8,07	10,31			2	31,3	40	0,03	yes	only species determination
A. aquaticus	AA135	AA_BE29_190321_04	11,4	300	15	0,6	8	1,7	6,16	7,44			2	33,1	40	0,027	yes	H8
A. aquaticus	AA136	AA_BO15_190329_06	11,4	300	16	0,6	8	1,7	6,34	8,75			2	29,0	40	0,032	yes	H2
A. aquaticus	AA137	AA_BE31_190322_05	11,3	300	18	0,6	8	1,7	7,34	9,34			2	31,4	40	0,023	yes	only species determination
A. aquaticus	AA138	AA_BE31_200427_10	7,8	300	19	0,6	8	1,7	8,89	12,25			2	29,0	40	0,024	yes	H6
A. aquaticus	AA139	AA_BE29_190321_02	11,1	300	20	0,6	8	1,7	7,22	9,85			2	29,3	40	0,027	yes	H8
A. aquaticus	AA140	AA_BE30_200427_20	7,7	300	21	0,6	8	1,7	9,65	13,24			2	29,1	40	0,026	yes	H6
A. aquaticus	AA141	AA_BE30_190321_10	10,3	278	22	0,6	8	1,7	5,88	7,75			2	30,3	40	0,033	yes	H2
A. aquaticus	AA142	AA_BO15_200422_18	6,7	250	23	0,5	8	1,4	7,38	10,84			2	27,2	40	0,041	yes	H2
A. aquaticus	AA143	AA_BE29_200427_16	6,6	250	25	0,5	8	1,4	6,53	8,36			2	31,2	40	0,029	yes	H6
A. aquaticus	AA144	AA_BO07_190402_02	9,1	245	27	0,5	8	1,4	6,39	9,79			2	26,1	40	0,022	yes	H12
A. aquaticus	AA145	AA_BO07_190402_03	9,1	250	1	0,5	10	1,4	5,77	7,33			2	31,5	40	0,021	yes	H12
A. aquaticus	AA146	AA_BO15_190329_07	8,7	235	2	0,5	10	1,4	4,34	5,83			2	29,8	40	0,036	yes	H1
A. aquaticus	AA147	AA_BE29_190321_06	8,6	240	3	0,5	10	1,4	6,61	8,30			2	31,9	40	0,027	yes	H1
A. aquaticus	AA148	AA_BE29_200427_13	5,9	235	4	0,5	10	1,4	5,61	6,75			2	33,3	40	0,042	yes	H1
A. aquaticus	AA149	AA_BO15_190329_04	8,2	222	5	0,5	10	1,4	6,55	8,92			2	29,4	40	0,032	yes	H1
A. aquaticus	AA150	AA_BO15_200422_14	5,6	243	6	0,5	10	1,4	6,91	9,28			2	29,8	40	0,063	yes	H8
A. aquaticus	AA151	AA_BO15_200422_11	5,3	200	7	0,5	10	1,1	6,72	9,10			2	29,5	40	0,031	yes	H8
A. aquaticus	AA152	AA_BE29_200427_15	5,2	200	8	0,5	10	1,1	5,11	7,69			2	26,6	40	0,033	yes	H2
A. aquaticus	AA153	AA_BE29_200427_14	5,2	218	9	0,5	10	1,1	5,62	7,71			2	29,1	40	0,045	yes	H6
A. aquaticus	AA154	AA_BO07_190402_05	7,4	208	10	0,5	10	1,1	4,01	5,67			2	28,3	40	0,022	yes	only species determination
A. aquaticus	AA155	AA_BE30_190321_06	6,9	192	11	0,5	10	1,1	4,94	7,48			2	26,4	40	0,03	yes	H8
A. aquaticus	AA156	AA_BE29_190321_10	6,7	187	12	0,5	10	1,1	5,27	8,23			2	25,6	40	0,022	yes	H7
A. aquaticus	AA157	AA_BE29_200427_17	4,6	195	13	0,5	10	1,1	3,81	4,45			2	34,2	40	0,045	yes	H6
A. aquaticus	AA158	AA_BO15_200422_12	4,2	179	14	0,5	10	1,1	4,43	9,33			2	19,0	40	0,075	yes	H1
A. aquaticus	AA159	AA_BO07_190402_06	5,6	148	15	0,5	10	1,1	1,89	2,27			2	33,3	40	0,019	yes	only species determination
A. aquaticus	AA160	AA_BO07_190402_04	3,9	103	16	0,5	10	1,1	1,03	1,26	0,4574	0,5313	2	33,0	40	0,029	yes	only species determination
A. aquaticus	AA161	AA_BO07_190402_10	3,7	102	18	0,5	10	1,1	3,10	4,29			2	28,8	40	0,021	yes	H1
A. aquaticus	AA162	AA_BO15_190329_10	3,5	93	19	0,5	10	1,1	2,22	3,21			2	27,7	40	0,027	yes	H8
A. aquaticus	AA163	AA_BE29_200427_12	2,3	72	20	0,5	10	1,1	3,81	5,84			2	26,1	40	0,024	yes	H17
A. aquaticus	AA164	AA_BO07_190402_09	2,6	72	21	0,5	10	1,1	0,39	0,51	1,3577	1,855	2	29,3	40	0,07	yes	only species determination
P. coxalis	PC1	PC_BE20_190319_05	20,4	500	22	1	10	2,8	7,56	10,46			2	28,9	40	0,038	yes	H1
P. coxalis	PC2	PC_BO26_190404_10	17,8	500	23	1	10	2,8	8,19	9,76			2	33,6	40	0,026	yes	H5
P. coxalis	PC3	PC_BO26_190404_01	15,5	400	25	0,8	10	2,2	8,21	11,40			2	28,8	40	0,025	yes	only species determination
P. coxalis	WPC1	PC_BE20_190319_09	10,6	300	27	0,6	10	1,7	11,08	13,25			2	33,5	40	0,024	yes	H1
P. coxalis	PC5	PC_BE21_190325_02	13,6	381	1	0,8	1	2,2	4,59	5,83			2	31,5	40	0,019	yes	H1
P. coxalis	PC6	PC_BO26_190404_02	13,5	378	2	0,8	1	2,2	1,03	1,35	3,0455	3,5633	2	34,2	40	0,023	yes	H5
P. coxalis	PC7	PC_BE21_190325_04	13,4	375	3	0,8	1	2,2	6,72	8,12			2	33,1	40	0,019	yes	H1
P. coxalis	PC8	PC_BE20_190319_06	12,7	300	4	0,6	1	1,7	6,65	8,12			2	32,8	40	0,02	yes	H1
P. coxalis	PC9	PC_BE20_190319_10	11,8	300	5	0,6	1	1,7	6,54	8,36			2	31,3	40	0,019	yes	H4
P. coxalis	PC10	PC_BE20_190319_01	11,7	300	6	0,6	1	1,7	1,89	4,42			2	17,1	40	0,112	yes	H4
P. coxalis	PC11	PC_BE20_190319_03	11,3	300	7	0,6	1	1,7	7,23	8,94			2	32,3	40	0,022	yes	only species determination
P. coxalis	PC12	PC_BE20_190319_02	11,1	300	8	0,6	1	1,7	7,49	8,98			2	33,4	40	0,024	yes	H1
P. coxalis	PC13	PC_BE20_190319_07	11,1	300	9	0,6	1	1,7	6,44	8,06			2	31,9	40	0,019	yes	H1
P. coxalis	PC14	PC_BO26_190404_03	10,9	300	10	0,6	1	1,7	8,84	11,04			2	32,0	40	0,02	yes	H5
P. coxalis	PC15	PC_BO23_190401_02	10,8	300	11	0,6	1	1,7	5,66	6,52			2	34,7	40	0,025	yes	H1
P. coxalis	PC16	PC_BO31_190405_02	10,7	300	12	0,6	1	1,7	9,10	11,67			2	31,2	40	0,033	yes	H4
P. coxalis	PC17	PC_BO23_190401_05	8,6	241	13	0,5	1	1,4	0,94	1,81			2	20,8	40	0,032	yes	H5
P. coxalis	PC18	PC_BE21_190325_01	8,6	240	14	0,5	1	1,4	7,75	10,22			2	30,3	40	0,024	yes	H1
P. coxalis	PC19	PC_BO23_190401_09	8,6	226	15	0,5	1	1,4	5,68	7,68			2	29,6	40	0,015	yes	H1
P. coxalis	PC20	PC_BO31_190405_01	8,6	240	16	0,5	1	1,4	3,34	4,96			2	26,9	40	0,024	yes	H4
P. coxalis	PC21	PC_BE21_190325_03	9,3	270	18	0,6	1	1,7	8,36	10,50			2	31,9	40	0,033	yes	H1
P. coxalis	PC22	PC_BE21_190325_05	9,2	271	19	0,6	1	1,7	6,29	7,79			2	32,3	40	0,035	yes	H1
P. coxalis	PC23	PC_BO23_190401_04	9,1	260	20	0,6	1	1,7	6,58	8,30			2	31,7	40	0,027	yes	H1
P. coxalis	PC24	PC_BO26_190404_09	9,0	264	21	0,6	1	1,7	5,95	7,92			2	30,1	40	0,031	yes	H5
P. coxalis	PC25	PC_BO23_190401_07	9,0	262	22	0,5	1	1,4	8,03	9,94			2	32,3	40	0,076	yes	H1
P. coxalis	PC26	PC_BO20_190329_02	9,0	258	23	0,5	1	1,4	8,07	9,96			2	32,4	40	0,037	yes	H1
P. coxalis	PC27	PC_BO26_190404_08	8,7	258	25	0,5	1	1,4	8,65	11,39			2	30,4	40	0,037	yes	H5
P. coxalis	PC28	PC_BE21_190325_08	8,7	251	27	0,5	1	1,4	8,46	11,36			2	29,8	40	0,03	yes	H1

P. coxalis	PC29	PC_BO23_190401_10	8,5	200	1	0,5	11	1,1	4,62	5,69	3	32,5	40	0,067	yes	only species determination
P. coxalis	PC30	PC_BO23_190401_01	8,0	200	2	0,5	11	1,1	8,98	11,08	3	32,4	40	0,024	yes	H1
P. coxalis	PC31	PC_BO31_190405_09	7,6	200	3	0,5	11	1,1	5,89	6,96	3	33,9	40	0,025	yes	H4
P. coxalis	PC32	PC_BO23_190401_06	7,4	200	4	0,5	11	1,1	8,85	11,56	3	30,6	40	0,025	yes	H1
P. coxalis	PC33	PC_BO07_190402_06	6,8	190	5	0,5	11	1,1	8,02	9,79	3	32,8	40	0,036	yes	H1
P. coxalis	PC34	PC_BE21_190325_09	6,7	188	6	0,5	11	1,1	8,75	11,23	3	31,2	40	0,025	yes	H1
P. coxalis	PC35	PC_BO26_190404_04	6,6	184	7	0,5	11	1,1	8,67	10,71	3	32,4	40	0,027	yes	H5
P. coxalis	PC36	PC_BO20_190329_01	6,1	171	8	0,5	11	1,1	8,58	10,73	3	32,0	40	0,04	yes	H1
P. coxalis	PC37	PC_BO07_190402_05	5,9	166	9	0,5	11	1,1	8,83	10,68	3	33,1	40	0,024	yes	H1
P. coxalis	PC38	PC_BO17_190329_01	5,9	165	10	0,5	11	1,1	8,50	11,42	3	29,8	40	0,03	yes	H1
P. coxalis	PC39	PC_BO17_190329_02	5,6	157	11	0,5	11	1,1	7,57	9,18	3	33,0	40	0,032	yes	H1
P. coxalis	PC40	PC_BO31_190405_08	5,5	155	12	0,5	11	1,1	8,77	11,52	3	30,5	40	0,035	yes	H4
P. coxalis	PC41	PC_BO31_190405_05	5,3	148	13	0,5	11	1,1	8,90	10,43	3	34,1	40	0,03	yes	only species determination
P. coxalis	PC42	PC_BE21_190325_07	5,2	146	14	0,5	11	1,1	9,04	10,70	3	33,8	40	0,023	yes	only species determination
P. coxalis	PC43	PC_BO26_190404_07	5,1	142	15	0,5	11	1,1	11,49	13,42	3	34,3	40	0,032	yes	only species determination
P. coxalis	PC44	PC_BO17_190329_04	4,6	129	16	0,5	11	1,1	10,54	12,91	3	32,6	40	0,031	yes	H1
P. coxalis	PC45	PC_BO31_190405_06	4,4	123	18	0,5	11	1,1	10,70	13,61	3	31,5	40	0,032	yes	H4
P. coxalis	PC46	PC_BO31_190405_07	4,3	120	19	0,5	11	1,1	9,63	11,46	3	33,6	40	0,026	yes	H4
P. coxalis	PC47	PC_BO07_190402_04	3,5	99	20	0,5	11	1,1	10,60	14,39	3	29,5	40	0,043	yes	H1
P. coxalis	PC48	PC_BO20_190329_03	2,7	74	21	0,5	11	1,1	10,00	13,34	3	30,0	40	0,035	yes	H1
P. coxalis	PC49	PC_BO17_190329_06	2,6	73	22	0,5	11	1,1	7,22	8,33	3	34,7	40	0,028	yes	only species determination
P. coxalis	PC50	PC_BO31_190405_04	2,6	72	23	0,5	11	1,1	11,76	15,82	3	29,7	40	0,036	yes	H4
P. coxalis	PC51	PC_BO17_190329_03	2,4	68	25	0,5	11	1,1	9,60	12,34	3	31,1	40	0,031	yes	H1
P. coxalis	PC52	PC_BO17_200422_09	15,2	500	27	1	11	2,8	8,72	10,89	3	32,0	40	0,034	yes	H1
P. coxalis	PC53	PC_BO17_200422_08	12,9	500	1	1	8	2,8	4,32	6,27	3	27,5	40	0,18	yes	H1
P. coxalis	PC54	PC_BO17_200422_07	10,5	400	2	0,8	8	2,2	8,89	10,76	3	33,1	40	0,037	yes	H1
P. coxalis	PC55	PC_BO17_200422_15	9,6	378	3	0,8	8	2,2	9,43	12,01	3	31,4	40	0,046	yes	H1
P. coxalis	PC56	PC_BO17_200422_20	8,9	350	4	0,7	8	1,9	10,12	12,89	3	31,4	40	0,038	yes	H1
P. coxalis	PC57	PC_BO17_200422_19	8,8	350	5	0,7	8	1,9	9,56	12,02	3	31,8	40	0,037	yes	H1
P. coxalis	PC58	PC_BO17_200422_11	8,6	334	6	0,7	8	1,9	9,63	12,12	3	31,8	40	0,035	yes	H1
P. coxalis	PC59	PC_BE21_200415_26	8,3	339	7	0,7	8	1,9	10,40	13,59	3	30,6	40	0,048	yes	H1
P. coxalis	PC60	PC_BO27_200416_04	8,1	349	8	0,7	8	1,9	9,52	11,95	3	31,9	40	0,112	yes	H1
P. coxalis	PC61	PC_BE20_200415_17	8,0	300	9	0,6	8	1,7	9,72	12,49	3	31,1	40	0,034	yes	H1
P. coxalis	PC62	PC_BO17_200422_18	8,0	300	10	0,6	8	1,7	11,44	14,54	3	31,5	40	0,045	yes	H1
P. coxalis	PC63	PC_BE21_200415_23	7,5	300	11	0,6	8	1,7	8,96	11,29	3	31,8	40	0,038	yes	H1
P. coxalis	PC64	PC_BO17_200422_12	7,1	299	12	0,6	8	1,7	8,09	10,33	3	31,3	40	0,054	yes	H1
P. coxalis	PC65	PC_BO31_200416_19	6,8	292	13	0,6	8	1,7	3,25	4,94	3	26,4	40	0,47	no	only species determination
P. coxalis	PC66	PC_BO25_200420_01	6,5	280	14	0,6	8	1,7	10,45	13,70	3	30,5	40	0,05	yes	H1
P. coxalis	PC67	PC_BO17_200422_14	6,5	279	15	0,6	8	1,7	9,56	11,55	3	33,1	40	0,036	yes	H1
P. coxalis	PC68	PC_BE21_200415_25	6,4	256	16	0,5	8	1,4	8,99	11,86	3	30,3	40	0,027	yes	H1
P. coxalis	PC69	PC_BO17_200422_16	6,2	250	18	0,5	8	1,4	10,58	12,56	3	33,7	40	0,032	yes	H1
P. coxalis	PC70	PC_BO27_200416_01	5,9	253	19	0,5	8	1,4	9,39	12,40	3	30,3	40	0,07	yes	H5
P. coxalis	PC71	PC_BO17_200422_21	5,7	246	20	0,5	8	1,4	9,64	12,42	3	31,1	40	0,036	yes	only species determination
P. coxalis	PC72	PC_BO23_200420_22	5,7	246	21	0,5	8	1,4	10,32	13,76	3	30,0	40	0,039	yes	H1
P. coxalis	PC73	PC_BO17_200422_17	5,7	244	22	0,5	8	1,4	10,39	12,64	3	32,9	40	0,046	yes	only species determination
P. coxalis	PC74	PC_BE21_200415_24	5,6	239	23	0,5	8	1,4	9,81	13,45	3	29,2	40	0,036	yes	H1
P. coxalis	PC75	PC_BO17_200422_13	5,3	200	25	0,5	8	1,1	9,71	12,59	3	30,9	40	0,044	yes	H1
P. coxalis	PC76	PC_BE21_200415_15	5,2	189	27	0,5	8	1,1	9,29	12,87	3	28,9	40	0,046	yes	H1
P. coxalis	PC77	PC_BO26_200416_14	5,0	200	1	0,5	1	1,1	6,30	7,48	3	33,7	40	0,026	yes	only species determination
P. coxalis	PC78	PC_BE21_200415_13	5,0	200	2	0,5	1	1,1	8,22	10,27	3	32,0	40	0,021	yes	H1
P. coxalis	PC79	PC_BE20_200415_15	4,8	200	3	0,5	1	1,1	7,70	9,50	3	32,4	40	0,022	yes	H1
P. coxalis	PC80	PC_BE21_200415_19	4,8	200	4	0,5	1	1,1	7,05	8,63	3	32,7	40	0,038	yes	H1
P. coxalis	PC81	PC_BO26_200416_13	4,6	198	5	0,5	1	1,1	7,85	9,80	3	32,0	40	0,025	yes	H5
P. coxalis	PC82	PC_BO26_200416_15	4,4	191	6	0,5	1	1,1	6,86	8,25	3	33,2	40	0,036	yes	H6
P. coxalis	PC83	PC_BO31_200416_18	4,4	191	7	0,5	1	1,1	8,16	10,35	3	31,5	40	0,023	yes	only species determination
P. coxalis	PC84	PC_BE21_200415_18	4,4	191	8	0,5	1	1,1	9,05	11,83	3	30,6	40	0,028	yes	H1
P. coxalis	PC85	PC_BO16_200422_05	4,4	189	9	0,5	1	1,1	8,93	10,85	3	32,9	40	0,027	yes	H1
P. coxalis	PC86	PC_BO31_200416_16	4,4	187	10	0,5	1	1,1	9,42	11,66	3	32,3	40	0,037	yes	only species determination
P. coxalis	PC87	PC_BO26_200416_12	4,3	175	11	0,5	1	1,1	7,98	10,34	3	30,9	40	0,026	yes	only species determination
P. coxalis	PC88	PC_BO31_200416_21	4,3	173	12	0,5	1	1,1	11,42	14,87	3	30,7	40	0,04	yes	H4
P. coxalis	PC89	PC_BE20_200415_13	4,2	181	13	0,5	1	1,1	8,59	10,44	3	32,9	40	0,019	yes	H1
P. coxalis	PC90	PC_BE20_200415_16	4,2	179	14	0,5	1	1,1	8,28	9,91	3	33,4	40	0,028	yes	H1
P. coxalis	PC91	PC_BO23_200420_17	4,0	172	15	0,5	1	1,1	9,58	12,04	3	31,8	40	0,027	yes	H1
P. coxalis	PC92	PC_BO23_200420_15	4,0	170	16	0,5	1	1,1	9,59	11,57	3	33,2	40	0,023	yes	H1
P. coxalis	PC93	PC_BO21_200420_03	3,9	168	18	0,5	1	1,1	10,11	13,77	3	29,4	40	0,032	yes	H2
P. coxalis	PC94	PC_BO31_200416_11	3,8	165	19	0,5	1	1,1	9,71	11,85	3	32,8	40	0,036	yes	H4
P. coxalis	PC95	PC_BO27_200416_03	3,7	160	20	0,5	1	1,1	11,73	15,09	3	31,1	40	0,058	yes	H3

P. coxalis	PC96	PC_BO21_200420_02	3,7	159	21	0,5	1	1,1	9,99	12,66	3	31,6	40	0,03	yes	H1
P. coxalis	PC97	PC_BO23_200420_25	3,6	153	22	0,5	1	1,1	11,52	15,54	3	29,7	40	0,058	yes	H1
P. coxalis	PC98	PC_BO31_200416_24	3,4	139	23	0,5	1	1,1	10,09	12,16	3	33,2	40	0,031	yes	H4
P. coxalis	PC99	PC_BO23_200420_21	3,4	145	25	0,5	1	1,1	10,07	13,62	3	29,6	40	0,031	yes	H1
P. coxalis	PC100	PC_BO31_200416_15	3,3	139	27	0,5	1	1,1	13,66	17,86	3	30,6	40	0,045	yes	only species determination
P. coxalis	PC101	PC_BO26_200416_16	3,3	140	1	0,5	10	1,1	11,20	13,68	3	32,7	40	0,035	yes	H5
P. coxalis	PC102	PC_BO31_200416_25	3,3	137	2	0,5	10	1,1	8,54	10,66	3	32,0	40	0,03	yes	only species determination
P. coxalis	PC103	PC_BE20_200415_14	3,1	132	3	0,5	10	1,1	9,12	11,19	3	32,6	40	0,025	yes	only species determination
P. coxalis	PC104	PC_BO27_200416_02	3,0	131	4	0,5	10	1,1	9,46	11,73	3	32,3	40	0,024	yes	H3
P. coxalis	PC105	PC_BO23_200420_24	3,0	129	5	0,5	10	1,1	12,09	15,20	3	31,8	40	0,05	yes	H5
P. coxalis	PC106	PC_BO07_200421_07	2,9	126	6	0,5	10	1,1	12,58	15,59	3	32,3	40	0,04	yes	H1
P. coxalis	PC107	PC_BO26_200416_11	2,9	118	7	0,5	10	1,1	9,67	12,12	3	31,9	40	0,032	yes	H5
P. coxalis	PC108	PC_BO27_200416_06	2,9	110	8	0,5	10	1,1	9,59	11,94	3	32,1	40	0,028	yes	H3
P. coxalis	PC109	PC_BO26_200416_22	2,9	123	9	0,5	10	1,1	10,04	12,76	3	31,5	40	0,032	yes	only species determination
P. coxalis	PC110	PC_BO23_200420_16	2,9	117	10	0,5	10	1,1	10,23	13,19	3	31,0	40	0,03	yes	H5
P. coxalis	PC111	PC_BO27_200416_05	2,8	121	11	0,5	10	1,1	10,84	12,68	3	34,2	40	0,026	yes	H3
P. coxalis	PC112	PC_BO23_200420_23	2,8	120	12	0,5	10	1,1	8,01	10,59	3	30,3	40	0,051	yes	H1
P. coxalis	PC113	PC_BO26_200416_18	2,6	112	13	0,5	10	1,1	4,75	5,68	3	33,4	40	0,032	yes	H5
P. coxalis	PC114	PC_BO27_200416_08	2,3	97	14	0,5	10	1,1	8,29	10,10	3	32,8	40	0,024	yes	H1
P. coxalis	PC115	PC_BE20_200415_12	2,3	96	15	0,5	10	1,1	7,07	8,42	3	33,6	40	0,019	yes	H1
P. coxalis	PC116	PC_BE20_200415_11	2,1	90	16	0,5	10	1,1	7,54	8,81	3	34,3	40	0,031	yes	H1
A. aquaticus		AA_BE29_190321_08								only COI sequences						H6
A. aquaticus		AA_BE29_190321_09								only COI sequences						H1
A. aquaticus		AA_BE30_190321_04								only COI sequences						H1
A. aquaticus		AA_BE30_200427_21								only COI sequences						H1
A. aquaticus		AA_BE30_200427_22								only COI sequences						H1
A. aquaticus		AA_BE30_200427_23								only COI sequences						H1
A. aquaticus		AA_BE30_200427_24								only COI sequences						H1
A. aquaticus		AA_BE30_200427_25								only COI sequences						H1
A. aquaticus		AA_BE31_200427_08								only COI sequences						H1
A. aquaticus		AA_BE31_200427_13								only COI sequences						H1
A. aquaticus		AA_BE31_200427_16								only COI sequences						H1
A. aquaticus		AA_BE31_200427_17								only COI sequences						H1
A. aquaticus		AA_BE31_200427_18								only COI sequences						H1
A. aquaticus		AA_BO00_190402_09								only COI sequences						H1
A. aquaticus		AA_BO00_200421_15								only COI sequences						H1
A. aquaticus		AA_BO00_200421_21								only COI sequences						H1
A. aquaticus		AA_BO00_200421_22								only COI sequences						H1
A. aquaticus		AA_BO00_200421_23								only COI sequences						H2
A. aquaticus		AA_BO00_200421_24								only COI sequences						H2
A. aquaticus		AA_BO00_200421_25								only COI sequences						H2
A. aquaticus		AA_BO07_200421_17								only COI sequences						H1
A. aquaticus		AA_BO07_200421_21								only COI sequences						H1
A. aquaticus		AA_BO07_200421_22								only COI sequences						H1
A. aquaticus		AA_BO07_200421_26								only COI sequences						H1
A. aquaticus		AA_BO11_200421_01								only COI sequences						H2
A. aquaticus		AA_BO11_200421_10								only COI sequences						H2
A. aquaticus		AA_BO11_200421_11								only COI sequences						H1
A. aquaticus		AA_BO11_200421_12								only COI sequences						H2
A. aquaticus		AA_BO11_200421_13								only COI sequences						H2
A. aquaticus		AA_BO11_200421_14								only COI sequences						H1
A. aquaticus		AA_BO11_200421_15								only COI sequences						H2
A. aquaticus		AA_BO15_190329_09								only COI sequences						H1
A. aquaticus		AA_BO15_200422_15								only COI sequences						H2
A. aquaticus		AA_BO15_200422_17								only COI sequences						H1
A. aquaticus		AA_BO15_200422_19								only COI sequences						H1
A. aquaticus		AA_BO15_200422_20								only COI sequences						H2
A. aquaticus		AA_BO15_200422_21								only COI sequences						H2
A. aquaticus		AA_BO15_200422_25								only COI sequences						H8
A. aquaticus		AA_BO16_200422_11								only COI sequences						H2
A. aquaticus		AA_BO16_200422_12								only COI sequences						H2
A. aquaticus		AA_BO16_200422_13								only COI sequences						H2
A. aquaticus		AA_BO24_190408_10								only COI sequences						H13
A. aquaticus		AA_BO24_200420_16								only COI sequences						H13
A. aquaticus		AA_BO24_200420_17								only COI sequences						H13
A. aquaticus		AA_BO24_200420_23								only COI sequences						H13

A. aquaticus	AA_BO24_200420_24	only COI sequences	H13
A. aquaticus	AA_BO27_190401_05	only COI sequences	H4
A. aquaticus	AA_BO27_200416_19	only COI sequences	H14
A. aquaticus	AA_BO27_200416_20	only COI sequences	H12
A. aquaticus	AA_BO27_200416_21	only COI sequences	H12
A. aquaticus	AA_BO27_200416_22	only COI sequences	H12
P. coxalis	PC_BE20_190319_04	only COI sequences	H1
P. coxalis	PC_BE20_190319_08	only COI sequences	H1
P. coxalis	PC_BE21_190325_10	only COI sequences	H1
P. coxalis	PC_BE21_190325_11	only COI sequences	H1
P. coxalis	PC_BE21_200415_14	only COI sequences	H1
P. coxalis	PC_BE21_200415_16	only COI sequences	H1
P. coxalis	PC_BE21_200415_17	only COI sequences	H1
P. coxalis	PC_BE21_200415_20	only COI sequences	H1
P. coxalis	PC_BE21_200415_22	only COI sequences	H1
P. coxalis	PC_BO17_190329_05	only COI sequences	H1
P. coxalis	PC_BO17_200422_10	only COI sequences	H1
P. coxalis	PC_BO20_190329_04	only COI sequences	H1
P. coxalis	PC_BO23_190401_03	only COI sequences	H1
P. coxalis	PC_BO23_200420_11	only COI sequences	H1
P. coxalis	PC_BO23_200420_12	only COI sequences	H1
P. coxalis	PC_BO23_200420_13	only COI sequences	H1
P. coxalis	PC_BO23_200420_19	only COI sequences	H1
P. coxalis	PC_BO26_190404_05	only COI sequences	H5
P. coxalis	PC_BO26_200416_19	only COI sequences	H5
P. coxalis	PC_BO26_200416_20	only COI sequences	H5
P. coxalis	PC_BO26_200416_21	only COI sequences	H5
P. coxalis	PC_BO26_200416_23	only COI sequences	H5
P. coxalis	PC_BO31_200416_14	only COI sequences	H4
P. coxalis	PC_BO31_200416_20	only COI sequences	H4
P. coxalis	PC_BO31_200416_23	only COI sequences	H4
P. coxalis	PC_BO31_200416_26	only COI sequences	H4

Tab. S3: Haplotype distribution for *A. aquaticus.* Given are numbers for each year and for both years together (indicated with grey background).

site	n	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19
BE29_19	**10**	**4**	**1**	0	0	0	**2**	**1**	**2**	0	0	0	0	0	0	0	0	0	0	0
BE29_20	**7**	**1**	**2**	0	0	0	**3**	0	0	0	0	0	0	0	0	0	0	**1**	0	0
BE29	**17**	**5**	**3**	0	0	0	**5**	**1**	**2**	0	0	0	0	0	0	0	0	**1**	0	0
BE30_19	**9**	**5**	**2**	0	0	0	**1**	0	**1**	0	0	0	0	0	0	0	0	0	0	0
BE30_20	**13**	**8**	**1**	0	0	0	**4**	0	0	0	0	0	0	0	0	0	0	0	0	0
BE30	**22**	**13**	**3**	0	0	0	**5**	0	**1**	0	0	0	0	0	0	0	0	0	0	0
BE31_19	**3**	**1**	0	0	0	0	**1**	0	**1**	0	0	0	0	0	0	0	0	0	0	0
BE31_20	**13**	**10**	**1**	0	0	0	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0
BE31	**16**	**11**	**1**	0	0	0	**3**	0	**1**	0	0	0	0	0	0	0	0	0	0	0
BO00_19	**8**	**4**	**2**	0	0	0	0	0	**1**	0	0	0	0	0	0	**1**	0	0	0	0
BO00_20	**15**	**7**	**6**	0	0	0	0	0	**1**	0	**1**	0	0	0	0	0	0	0	0	0
BO00	**23**	**11**	**8**	0	0	0	0	0	**2**	0	**1**	0	0	0	0	**1**	0	0	0	0
BO02_20	**3**	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0
BO07_19	**4**	**2**	0	0	0	0	0	0	0	0	0	0	**2**	0	0	0	0	0	0	0
BO07_20	**13**	**11**	**1**	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0
BO07	**17**	**13**	**1**	0	0	0	0	0	0	0	0	0	**3**	0	0	0	0	0	0	0
BO09_19	**1**	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BO11_20	**15**	**3**	**10**	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0	0	**1**
BO15_19	**9**	**5**	**3**	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0
BO15_20	**14**	**6**	**5**	0	0	0	0	0	**3**	0	0	0	0	0	0	0	0	0	0	0
BO15	**23**	**11**	**8**	0	0	0	0	0	**4**	0	0	0	0	0	0	0	0	0	0	0
BO16_19	**2**	**2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BO16_20	**11**	**6**	**5**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BO16	**13**	**8**	**5**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BO24_19	**9**	0	0	0	0	0	0	0	0	**2**	0	**1**	0	**6**	0	0	0	0	0	0
BO24_20	**16**	0	0	0	**2**	0	0	0	0	**3**	0	0	0	**10**	0	0	0	**1**	0	0
BO24	**25**	0	0	0	**2**	0	0	0	0	**5**	0	**1**	0	**16**	0	0	0	**1**	0	0
BO25_20	**5**	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**3**	**1**	0
BO27_19	**8**	0	0	0	**3**	**3**	0	0	0	0	0	0	**2**	0	0	0	0	0	0	0
BO27_20	**11**	0	0	0	**3**	**2**	0	0	0	0	**1**	0	**3**	0	**2**	0	0	0	0	0
BO27	**19**	0	0	0	**6**	**5**	0	0	0	0	**1**	0	**5**	0	**2**	0	0	0	0	0
Sum	199	78	39	1	8	5	13	1	10	5	2	1	8	16	2	2	1	5	1	1

Tab. S4: Results for the best partitions found by ASAP using K80 as a substitution model for *A. aquaticus* and *P. coxalis*.

species	Nb of species	asap-score	P-val (rank)	W (rank)	Treshold dist.
A. aquaticus	2	1,00	2.61e-03 (1)	5.09e-05 (1)	0,06937
	12	3,50	1.56e-01 (4)	1.22e-05 (3)	0,00536
	7	3,50	1.68e-01 (5)	1.43e-05 (2)	0,00984
	6	5,00	1.22e-01 (3)	3.47e-06 (7)	0,01164
	10	5,00	1.86e-01 (6)	7.90e-06 (4)	0,00804
	4	6,00	3.71e-01 (7)	3.98e-06 (5)	0,01348
	19	6,50	1.80e-02 (2)	4.46e-07 (11)	0,00089
	3	7,50	4.23e-01 (9)	3.49e-06 (6)	0,01804
	13	8,50	3.77e-01 (8)	1.25e-06 (9)	0,00267
	5	9,00	5.07e-01 (10)	2.46e-06 (8)	0,01255
P. coxalis	3	1,50	7.16e-04 (2)	1.37e-04 (1)	0,00968
	3	2,50	1.00e-05 (1)	1.57e-05 (4)	0,03579
	4	3,50	3.83e-02 (4)	1.79e-05 (3)	0,00676
	5	3,50	5.80e-02 (5)	7.67e-05 (2)	0,00482
	6	4,00	3.44e-02 (3)	1.52e-06 (5)	0,00193

Tab. S5: Different measures for genetic diversity for both species per sampling site. Diversity measures for ddRAD are: allelic richness (AR) and observed heterozygosity (Ho). Measures for COI are haplotype diversity (HDiv) and nucleotide diversity (NDiv). n is the number of specimens for each marker. Diversity was only calculated for sites with n > 5.)

species	site	status site	ddRAD			COI		
			n	AR	Ho	n	HDiv	NDiv
	BO00	restored (2020)	15	1,47	0,12	23	0,668	0,0085
	BE30	restored (2011)	18	1,46	0,13	22	0,606	0,0067
	BE29	restored (2012)	15	1,46	0,13	17	0,824	0,0234
	BO15	restored (2011)	16	1,45	0,13	23	0,648	0,0073
	BO24	near-natural	21	1,44	0,12	25	0,563	0,0212
A. aquaticus	BE31	near-natural	13	1,43	0,12	16	0,517	0,0066
	BO25	near-natural	7	1,42	0,13	5	0,700	0,0468
	BO16	restored (1993)	10	1,42	0,12	13	0,513	0,0009
	BO11	restored (2008)	8	1,39	0,12	15	0,543	0,0205
	BO07	restored (2011)	17	1,39	0,12	17	0,404	0,0085
	BO27	near-natural	16	1,38	0,11	19	0,790	0,0184
	BE20	waste-water free	15	1,45	0,14	15	0,248	0,0143
	BE21	waste-water free	16	1,45	0,14	22	0,000	0,0000
	BO17	restored (1993)	19	1,46	0,14	18	0,000	0,0000
P. coxalis	BO23	near-natural	16	1,48	0,14	20	0,268	0,0165
	BO26	near-natural	16	1,43	0,13	16	0,125	0,0012
	BO27	near-natural	7	1,41	0,14	7	0,667	0,0238
	BO31	near-natural	15	1,4	0,13	14	0,000	0,0000

Tab. S6: Summary statistics for all stacks settings for *A. aquaticus* and *P. coxalis* (Test) and for the final dataset (Final); loci limit = percentage of specimens required to have the loci, ma = minor allele frequency, H_O = observed heterozygosity, H_S = within population gene diversity, H_T = overall gene diversity, best K: K with lowest cross-entropy (median from all repetitions) in sNMF analysis.

species	Test stacks settings/ Final	stacks setting	loci limit	ma	# loci	H_O	H_S	H_T	F_{ST}	F_{IS}	best K
A. aquaticus	Test	m3 M2 N4 n3	l90	ma1	2806	0.1260	0.1553	0.1689	0.0963	0.1835	5
A. aquaticus	Test	m3 M2 N4 n2	l90	ma1	2661	0.1237	0.1529	0.1664	0.0973	0.1851	5
A. aquaticus	Test	m3 M3 N5 n4	l90	ma1	2642	0.1255	0.1548	0.1683	0.0952	0.1847	4
A. aquaticus	Test	m3 M3 N5 n3	l90	ma1	2598	0.1243	0.1533	0.1669	0.0963	0.1847	5
A. aquaticus	Test	m3 M4 N6 n4	l90	ma1	2536	0.1252	0.1542	0.1678	0.0958	0.1844	5
A. aquaticus	Test	m3 M4 N6 n5	l90	ma1	2520	0.1259	0.1551	0.1689	0.0962	0.1848	4
A. aquaticus	Test	m3 M5 N7 n5	l90	ma1	2452	0.1248	0.1539	0.1677	0.0962	0.1846	5
A. aquaticus	Test	m3 M5 N7 n6	l90	ma1	2444	0.1249	0.1541	0.1677	0.0959	0.1847	4
A. aquaticus	**Final**	m3 M2 N4 n3	**l90**	**ma1**	**3302**	**0.1227**	**0.1532**	**0.1666**	**0.0961**	**0.1928**	**5**
P. coxalis	Test	m3 M3 N5 n4	l90	ma1	11764	0.1416	0.1688	0.1906	0.1442	0.1651	5
P. coxalis	Test	m3 M4 N6 n5	l90	ma1	11750	0.1404	0.1673	0.1888	0.1448	0.1637	5
P. coxalis	Test	m3 M2 N4 n3	l90	ma1	11603	0.1387	0.1652	0.1865	0.1453	0.1624	5
P. coxalis	Test	m3 M5 N7 n6	l90	ma1	11589	0.1420	0.1694	0.1913	0.1442	0.1654	5
P. coxalis	Test	m3 M5 N7 n5	l90	ma1	11424	0.1411	0.1684	0.1901	0.144	0.1662	5
P. coxalis	Test	m3 M4 N6 n4	l90	ma1	11381	0.1406	0.1676	0.1892	0.1447	0.165	5
P. coxalis	Test	m3 M3 N5 n3	l90	ma1	11038	0.1384	0.1654	0.1868	0.145	0.1664	5
P. coxalis	Test	m3 M2 N4 n2	l90	ma1	9848	0.1344	0.1613	0.1822	0.1458	0.1661	5
P. coxalis	**Final**	**m3 M3 N5 n4**	**l90**	**ma1**	**12186**	**0.1402**	**0.168**	**0.1898**	**0.1455**	**0.168**	**5**

Tab. S7: Haplotype distribution for *P. coxalis.* Given are numbers for each year and for both years together (indicated with grey background).

site	n	H1	H2	H3	H4	H5	H6
BE20_19	**9**	**7**	0	0	**2**	0	0
BE20_20	**6**	**6**	0	0	0	0	0
BE20	**15**	**13**	0	0	**2**	0	0
BE21_19	**9**	**9**	0	0	0	0	0
BE21_20	**13**	**13**	0	0	0	0	0
BE21	**22**	**22**	0	0	0	0	0
BO07_19	**3**	**3**	0	0	0	0	0
BO07_20	**1**	**1**	0	0	0	0	0
BO07	**4**	**4**	0	0	0	0	0
BO16_20	**1**	**1**	0	0	0	0	0
BO17_19	**5**	**5**	0	0	0	0	0
BO17_20	**13**	**13**	0	0	0	0	0
BO17	**18**	**18**	0	0	0	0	0
BO20_19	**4**	**4**	0	0	0	0	0
BO21_20	**2**	**1**	**1**	0	0	0	0
BO23_19	**8**	**7**	0	0	0	**1**	0
BO23_20	**12**	**10**	0	0	0	**2**	0
BO23	**20**	**17**	0	0	0	**3**	0
BO25_20	**1**	**1**	0	0	0	0	0
BO26_19	**7**	0	0	0	0	**7**	0
BO26_20	**9**	0	0	0	0	**8**	**1**
BO26	**16**	0	0	0	0	**15**	**1**
BO27_20	**7**	**2**	0	**4**	0	**1**	0
BO31_19	**7**	0	0	0	**7**	0	0
BO31_20	**7**	0	0	0	**7**	0	0
BO31	**14**	0	0	0	**14**	0	0
Sum	**124**	**83**	**1**	**4**	**16**	**19**	**1**