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Unveiling the Genomic Landscape of *Pseudorasbora parva*, the Most Invasive Freshwater Fish Worldwide: A Key Step Towards Understanding Invasion Dynamics

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Abstract: Invasive species often defy theoretical expectations, successfully establishing and spreading despite reduced propagule pressure and limited genetic diversity. What genomic mechanisms underpin this paradox? How do adaptive processes and host–pathogen interactions shape invasion outcomes? And which genes drive resistance and modulate pathogen virulence? Here, we address these questions using a model of co-invasion: the Asian topmouth gudgeon (*Pseudorasbora parva*) and its fungal parasite the Rosette agent (*Sphaerothecum destruens*), a system with profound ecological and economic consequences. Here by (1) mapping the reads obtained by Illumina sequencing on a previously deposited *P. parva* genome from Germany, (2) identifying SNPs and (3) creating a consensus sequence, we generated the first whole genome of an invasive *P. parva* population in France and compared it to a German population to explore patterns of genetic diversity, local adaptation, and potential signatures of pathogen resistance. Despite historical bottlenecks, our results reveal unexpectedly high levels of genomic diversity between these invasive populations. We identify candidate loci linked to immune function and provide insights into the evolutionary dynamics of co-introduction. These findings offer a rare window into how invasive species maintain adaptability and how pathogens may co-evolve during range expansion. Beyond advancing our understanding of invasion biology, the genomic resources generated here pave the way for translational approaches, including the development of genome-editing strategies aimed at mitigating the impact of invasive species and their associated pathogens. This work marks a critical step toward unraveling the complex interplay between genetics, ecology, and evolution in biological invasions.

Keywords: biological invasion; aquatic; genetics; cyprinid; whole genome sequencing

Key Contribution: This study presents the first genome assembly of *Pseudorasbora parva;* a highly invasive fish; in France. Comparison with a german reference revealed substantial genomic diversity; including signatures of selection notably affecting genes involved in immune system function.

Academic Editor: Jose Martin Pujolar

Received: 6 May 2025
Revised: 6 June 2025
Accepted: 12 June 2025
Published: 19 June 2025

Citation: Combe, M.; Deremarque, T.; Givens, J.; Gozlan, R.E. Unveiling the Genomic Landscape of *Pseudorasbora parva*, the Most Invasive Freshwater Fish Worldwide: A Key Step Towards Understanding Invasion Dynamics. *Fishes* **2025**, *10*, 297. https://doi.org/10.3390/fishes10060297

1. Introduction

Biological invasions represent a significant ecological challenge [1,2], driven by globalization, trade, and expanding transportation networks [3]. These invasions disrupt ecosystems through the destabilization of trophic networks [4,5], competition for resources and habitats [6,7], predation on native species [8], and the transmission of novel pathogens [9].

Despite theoretical expectations that small propagule pressure and reduced genetic diversity should constrain introduced populations [10–14], many invasive species succeed in establishing and spreading. This paradox raises critical questions: How do genetic variability and adaptive processes facilitate invasion? What roles do genomic mechanisms and host–pathogen dynamics play in shaping invasion outcomes? Which are the genes involved in pathogen resistance and how do they modulate fitness and virulence? Recent studies reveal that invasions are frequently characterized by complex genetic processes, such as multiple introductions and admixture events, which amplify genetic diversity and adaptive potential [15,16]. Understanding these dynamics, particularly the genetic architecture of both native and invasive populations, is essential for decoding the evolutionary and ecological drivers of invasion success [17].

The topmouth gudgeon *Pseudorasbora parva* (described first by Temminck & Schlegel in 1846) [18], a small freshwater cyprinid, provides a compelling model for addressing these questions. Native to East Asia (including Eastern China, Taiwan, Korea, and Japan) *P. parva* has undergone a pan-continental invasion over the last six decades, facilitated by human-mediated introductions and remarkable adaptability (e.g., from continental climates to tropical ones) [19,20]. Initially, *P. parva* was accidentally released outside its native range through the exchanges of farmed Chinese carp between China and former countries of the Soviet Bloc [21]. During the 1960s, several introductions of *P. parva* took place in countries all around the Black Sea, followed by further introductions in the 1980s in Eurasia and North Africa. After these initial steps of human-made introductions, local colonization of entire river networks occurred across major European rivers to the Middle East [22]. Life-history traits, such as early maturity (1 year) and nest-guarding behavior, underpin its rapid establishment and spread [19,21,23]. However, its invasion success also correlates with its ability to harbor high genetic diversity, supporting adaptation to novel environments [15]. Moreover, *P. parva* acts as an asymptomatic carrier of the rosette agent *Sphaerothecum destruens*, a fungus-like pathogen with severe impacts on native freshwater biodiversity and aquaculture [24]. Due to its significant threat to biodiversity, ecological and economic impact, and its co-invasive nature, this host–parasite system is a critical focus of study within the field of invasion biology [25].

Despite significant progress in understanding its genetic structure through mitochondrial, nuclear, and SNP-based studies and phylogeography of both native and invasive populations, the complete genome of different *P. parva* populations has yet to be sequenced. The current studies suggest the existence of two genetic lineages within native and non-native populations [26–29]. Such a pattern was further confirmed by a study using 13,785 single nucleotide polymorphisms (SNPs) in Slovakian and Turkish introduced populations [30]. Recent advancements have shed light on the genetic architecture of *P. parva* using comprehensive genotyping approaches. A total of 858 DNA samples from 746 individuals were analyzed for SNPs through genotyping-by-sequencing (GBS), employing genomic DNA digestion with PstI. By integrating these genomic datasets with Approximative Bayesian Computation (ABC) models and machine learning algorithms, the genetic structure and gene flow of *P. parva* across 21 discrete sites in Asia, spanning 16 river catchments in its historical Chinese native range, as well as Japan, Tibet, and Southeast China, were characterized. The findings revealed that the present genetic landscape of *P. parva* in its native range has been shaped by successive waves of gene flow originating from southern and northern Chinese populations. These results underscore the genetic diversity observed in invasive populations that arises from multiple introduction events involving admixed source populations [29].

The disentangling of the *P. parva* genome would align with recent de novo sequencing of highly invasive aquatic species such as *Rana catesbeiana* [31], *Oreochromis aureus* [32],

and *Procambarus clarkii* [33]. Previous studies that aimed at resolving *P. parva* population genomic complexity relied on limited markers such as mitochondrial DNA [26,28], microsatellites loci [28], or enzyme restriction-associated sites [29,30,34]. While these markers are commonly exploited to characterize invasion pathways [35], admixture profiles [36], and diversity patterns between populations [37], they are gradually being replaced or complemented by whole-genome (re)sequencing [38,39]. Despite displaying higher costs and requiring more complex data processing, WGS offers improved resolution in systems with weak population structures, enhances the identification of low-frequency alleles [40], and searches for potential local adaptation within functional loci. Importantly, the description of the complete genome of *P. parva* would enable deeper insights into the genetic mechanisms underlying its invasion success, including gene-environment interactions, and the evolutionary dynamics of co-introduction. It would also provide an unprecedented opportunity to assess the genetic basis of distinct morphotypes observed in its native range and their potential roles as ecotypes or cryptic species. Additionally, sequencing the complete genome of *P. parva* would enable the identification of specific genes associated with resistance to its fungal parasite, *S. destruens*. This would include understanding the regulation of these genes, their evolutionary dynamics during the invasion process, and their impact on the fitness of the fungal parasite. Such genomic data could facilitate the development of CRISPR-like molecular scissors to target and disrupt host resistance genes, potentially eradicating both the carrier host and the infectious agent.

Whilst the invasion of Germany and France occurred around the same period (late 1970s–early 1980s) from an initial invasion from China through Eastern Europe [21], the German population arose from successive introductions from Hungary and through Czechoslovakia [21] while the French population appears to find its origin through a single introduction coming from an Albanian population [19,41]. In spite of similar haplotype diversity within mitochondrial DNA [28] and similar heterozygosity of microsatellite [27], German and French *P. parva* populations exhibited different genetic clusters [27]. The genetic diversity of *P. parva* populations in Germany is poorly documented, contrasting with the thoroughly studied French populations [30,34]. Indeed, french *P. parva* populations showed a homogeneous gene pool, a reduced diversity compared to native populations, and a distinct clustering compared to other invasive populations. Here, we present the whole-genome sequence of *P. parva* from an invasive population in France and perform a comparative analysis with a German population. Despite historical bottlenecks, we aim to investigate whether invasive populations of *P. parva* retain unexpectedly high levels of genetic diversity. We also seek to identify candidate functional loci potentially involved in local adaptation and invasion success. These analyses are intended to provide a critical foundation for exploring the genomic basis of co-invasion and to open new avenues for understanding how evolutionary processes shape the spread and ecological impact of invasive species.

2. Materials and Methods

2.1. Sampling

Fish was collected by electrofishing in June 2022 in La Claise River, close to the city Mézières-en-Brennes (latitude 46.824535, longitude 1.255693), by "La Fédération de l'Indre pour la Pêche et la Protection du Milieu Aquatique (FDAAPPMA36)" (Figure 1A,B). *P. parva* fish collected was taxonomically identified as our group has a long experience in *P. parva* identification in its native and invasive range, also working in close collaboration with the Museum of National History in Paris. The fish was immediately euthanized with an overdose of tricaine. Dr. Combe and Prof. Gozlan are trained for animal (fish) manipulation and are notably allowed to practice fish euthanasia. The ANR DRIVE project received the

Nagoya agreement N°TREL2302365S/661 from the "Ministère de la Transition Écologique et de la Cohésion des Territoires" as well as an internationally recognized certificate of compliance constituted from information on the permit or its equivalent made available to the Access and Benefit-sharing Clearing-house N°ABSCH-IRCC-FR-263712-1.

Figure 1. (**A**) Map of the sampling location of *P. parva* in La Claise River, indicated by a red dot close to the city Mézières-en-Brennes (latitude 46.824535, longitude 1.255693), northern France, in June 2022. (**B**) The fish were collected by electrofishing by "La Fédération de l'Indre pour la Pêche et la Protection du Milieu Aquatique (FDAAPPMA36)". The fish collected were immediately euthanized with an overdose of tricaine.

2.2. DNA Extraction and Sequencing

Genomic DNA was extracted from fish tissues (tail fin) collected from a single *P. parva* individual and preserved in liquid nitrogen. We used the Qiagen Blood and Cell Culture DNA Kit with Genomic Tips 20/G following the manufacturer's recommendations for extracting gDNA from tissue samples, with some more specific steps such as (i) the use of 60 mg of tissues, (ii) the crushing of the tissues in liquid nitrogen, (iii) a centrifugation step for 1 h at 15,000× *g* after the isopropanol precipitation, and (iv) the elution step in 100 µL. gDNA quantification was performed with Qubit High Sensitivity (Thermofisher Scientific, Villebon-sur-Yvette, France) and the concentration used for sequencing was 48.2 ng/µL.

Our initial aim was to obtain a de novo genome assembly using Nanopore and Illumina technologies (GenoScreen *Innovative Genomics*, https://www.genoscreen.fr/en/genoscreen/about, URL accessed on 20 February 2023). However, since the two Nanopore sequencing runs failed (coverage < 6×), we were not able to use Nanopore reads. Thus, we changed our strategy to an approach by mapping the reads obtained with Illumina only on a previously deposited *P. parva* genome (reference ASM2467924v1), followed by the identification of single nucleotide polymorphisms (SNPs) and then the creation of a consensus sequence integrating previously identified SNPs. Library preparation was performed with the Illumina DNA Prep kit (https://emea.illumina.com/products/by-type/sequencing-kits/library-prep-kits/illumina-dna-prep.html, URL accessed on 26 February 2023) following the manufacturer's recommendations, except the equimolar pool preparation that was performed using an optimized protocol by GENOSCREEN. Briefly, 100 ng of gDNA were fragmented and ligated to the sequencing adapters p5 and p7, and to the index adapters i5 and i7, using the following PCR program: 68 °C for 3 min, 98 °C for 3 min, followed by 5 cycles of 98 °C for 45 s, 62 °C for 30 s, and 68 °C for 2 min. The PCR was finalized with a 1 min extension at 68 °C. After amplification, the fragments of size suitable for paired-end sequencing (around 500 bp) were selected using magnetic beads. The quality control carried out on the final library obtained consisted of (i) a qualitative control by capillary electrophoresis on a High-Sensitivity chip (Agilent, Santa Clara, CA, USA) using Agilent High-Sensitivity DNA kit following the manufacturer's recommendations to validate its size distribution and (ii) a quantitative control (in ng/µL) by a fluorimetric assay with SybrGreen (Thermofisher Scientific, France) using an internal protocol developed by GenoScreen (Lille, France). Paired-end sequencing (2 × 150 bp) was performed using a novaseq6000 platform and an S4 flowcell.

2.3. Bio-Informatic Analysis

Illumina QC was performed using fastQC (v0.12.1). Filtering was performed using TrimGalore! (v0.6.10) (https://github.com/FelixKrueger/TrimGalore, URL accessed on 5 March 2023) in order to remove the adapter sequence and prinseq-lite (v0.20) to filter sequences based on quality [42]. Those steps allowed us to obtain excellent quality reads (>Q30) to perform the downstream analysis. Alignment of the Illumina reads on the reference sequence was performed using bowtie2 (v2.4.5) with local parameters [43]. The reference used for the mapping was composed of the 81 contigs from *P. parva* ASM2467924v1 (including 25 chromosomes, 1 mitochondrial sequence, and 55 unplaced scaffolds). The variant calling was performed using bcftools call with the consensus caller. The indel were left-aligned to eliminate ambiguity using the bcftools norm, and then the variants were filtered using a bcftools command described in the Supplementary Materials (Supplementary Table S1) [44]. This filtering retained only biallelic SNVs with high variant calling quality (QUAL > 30), high mapping quality (MQ > 30), and adequate read depth (10 < DP < 100). Also, SNVs that were within 5 bases of an InDel were filtered out as the alignment around InDels can be problematic and produce false positives. Finally, the consensus sequence was generated using bcftools consensus by applying previously detected variants on the reference genome. The expected size of the *P. parva* genome was about 1.229 Gb.

2.4. Variant Analysis

The snpEff software (v5.2f) [45] was exploited to annotate and characterize the SNPs identified by variant calling uncovering the genomic region impacted by each SNP and its associated variant type. The species database required to run snpEff was generated using *P. parva* ASM2467924v1 annotation of CDSs, genes, and proteins. snpEff annotation allowed for the identification of synonymous and nonsynonymous variants while EggLib

(v3.3.5) [46] was used to determine the number of synonymous and nonsynonymous sites within every gene. Genes exhibiting a combined number of synonymous and nonsynonymous variants < 5 were discarded from further analysis. The remaining data enabled the computation of pN (ratio of nonsynonymous variants/nonsynonymous sites) and pS (ratio of synonymous variants/synonymous sites), extrapolating the pN/pS ratios for each gene. Genes with pN/pS < 1 are generally interpreted as being under neutral or purifying selection while genes with pN/pS > 1 may be subject to positive or diversifying selection. However, given the limited number of specimens included in this study, we have to consider the potential influence of genetic drift and geographical distance on the pN/pS ratios.

2.5. Gene Ontology Enrichment Analysis

Gene Ontology (GO) annotation of *P. parva* genes was recovered from NCBI RefSeq's Eukaryote Genome Annotation Pipeline for the ASM2467924v1 genome. GO annotation was available for 22,536 of the 26,297 *P. parva*'s protein-coding genes. GO enrichment analysis was performed on genes having a GO annotation available and displaying a pN/pS ratio > 1 using the enricher function of ClusterProfiler (v4.10.1) with standard parameters (pvalueCutoff = 0.05, pAdjustMethod = 'BH', qvalueCutoff = 0.2). The biomaRt package (v2.58.2) allowed us to obtain the GO descriptions matching the GO IDs identified by ClusterProfiler.

3. Results

3.1. Illumina Sequencing and Mapping

The Illumina paired-end (2 × 150 bp) sequencing showed 88.43% of bases with a quality score > Q30 which allowed us to obtain a sequencing depth > 50×. The Illumina sequencing generated 301,195,015 raw paired reads that contained 90,358,504,500 bases (90,358 Gb), representing a theoretical depth of 75×. After filtering we kept 259,897,752 reads that contained 71,557,874,897 bases (71,557 Gb), which represent 86.29% of the reads and 79.19% of the nucleotides sequenced, representing then a theoretical depth of 58×. By mapping these filtered reads, 99.08% of the reads were mapped on the reference genome *P. parva* ASM2467924v1, with an overall coverage of 98.79%. For the contigs, the lowest coverage was 76.07% and the highest was 100%. The observed range of mean coverage depth was comprised between 19× and 237× across most contigs, with two contigs having a mean depth of 1538× and 3507× while the mitochondrial chromosome had a mean depth of 3733×.

3.2. Variant Calling

Variant calling identified 8,797,589 high-confidence SNPs within the 1.2 Gb reference genome corresponding to 1 polymorphic position every 136 bp or 7331 SNP/Mbps. The genomic distribution of variants, considering both the entire genome and specifically exonic regions, was analyzed at the chromosome scale (Table 1) and in 1 Mbp windows (Figure 2). At a chromosomal level, SNP frequencies were relatively homogeneous across all chromosomes, both genome-wide (frequency ranging from 0.00528 for Chr2 to 0.00834 for Chr23) and within exonic regions (frequency ranging from 0.00015 for Chr2 to 0.00024 for Chr23). However, at a finer resolution, SNP distribution was more disparate with frequencies ranging from 0 (Chr22) to 0.0129 (Chr16) in the whole genome and from 0 (Chr11, Chr16, Chr20, and Chr22) to 0.00085 (Chr11) in exonic regions.

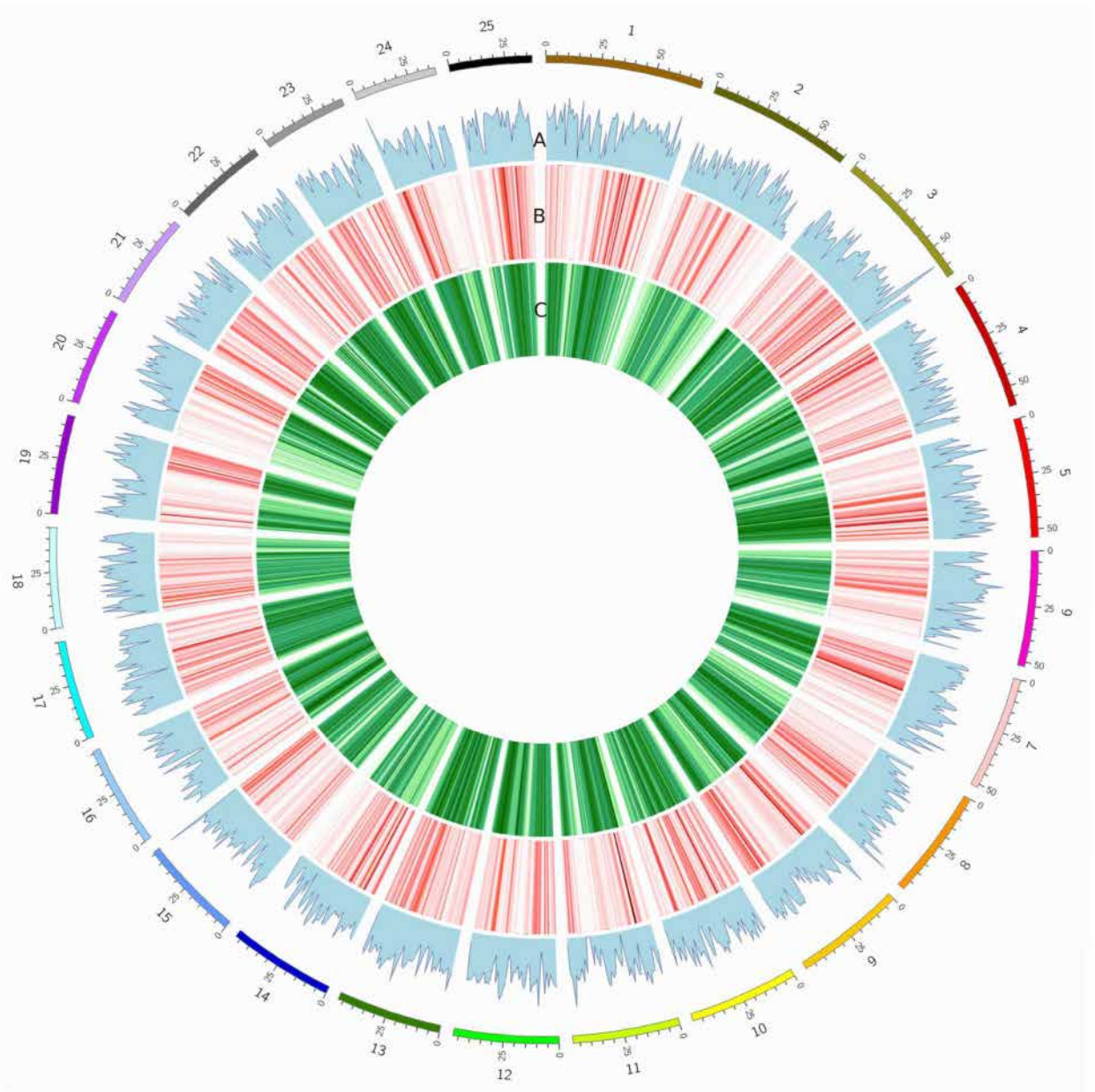

Figure 2. French *P. parva* genome characteristics after variant calling against the German reference genome. Circos diagram showing the following (outer to inner): (**A**) blue colored lines represent gene density (cumulative gene length per 1 Mb window); (**B**) heatmap of SNP frequency within exonic regions per 1 Mb window with color intensity reflecting SNP density, ranging from white (low density) to dark red (high density); (**C**) heatmap of SNP frequency within all the genomic regions per 1 Mb window with color intensity reflecting SNP density, ranging from white (low density) to dark blue (high density).

When considering the genomic region where SNPs were found, almost half of them (49.8%) were localized within intronic regions (Figure 3) and 97.8% of the SNPs were found within non-coding regions (INTRON, DOWNSTREAM, UPSTREAM, INTERGENIC, UTR 3 PRIME, and UTR 5 PRIME). SNPs impacting coding regions were found either in exons (2.1%) or in splice site regions (0.1%). Among the SNP-impacting exonic regions, 66% of them generated a synonymous variant while 34% created a missense variant.

Table 1. Genomic distribution of variants, considering both the entire genome and specifically exonic regions, analyzed at the chromosome scale.

Chromosome	Variant Frequency Genome-Wide	Variant Frequency in Exonic Regions
Chr1	0.00724	0.00018
Chr2	0.00528	0.00015
Chr3	0.00765	0.00020
Chr4	0.00723	0.00020
Chr5	0.00798	0.00021
Chr6	0.00595	0.00017
Chr7	0.00752	0.00020
Chr8	0.00680	0.00019
Chr9	0.00756	0.00022
Chr10	0.00760	0.00021
Chr11	0.00711	0.00016
Chr12	0.00790	0.00020
Chr13	0.00820	0.00023
Chr14	0.00546	0.00019
Chr15	0.00697	0.00019
Chr16	0.00769	0.00017
Chr17	0.00820	0.00021
Chr18	0.00657	0.00019
Chr19	0.00695	0.00019
Chr20	0.00569	0.00016
Chr21	0.00830	0.00022
Chr22	0.00772	0.00019
Chr23	0.00834	0.00024
Chr24	0.00737	0.00018
Chr25	0.00708	0.00022

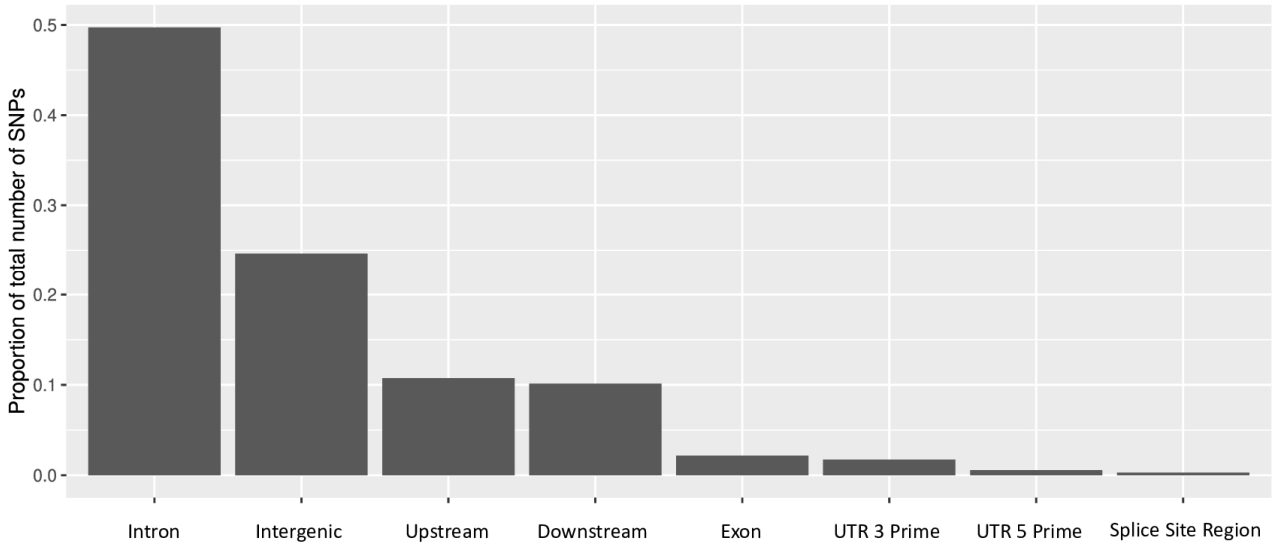

Figure 3. Statistics of SNPs detected in each type of genomic region.

3.3. pN/pS Ratios and GO Enrichment

After analyzing the impact of the SNP contained within the exonic region (either missense or synonymous), we computed the pN/pS ratio of each protein-coding gene with a combined number of missense and synonymous variants > 5. The mean pN/pS ratio was 0.227. Among the 26,297 annotated protein-coding genes, 529 genes displayed a pN/pS ratio > 1 accounting for 2% of all the protein-coding genes. Of those 529 genes, 373 had a GO annotation allowing for a GO enrichment against the 22,536 *P. parva* genes with a GO annotation. GO enrichment unveiled 13 terms (1 implied in cellular component, 6 in biological process, and 6 in molecular function) overrepresented in genes displaying a pN/pS ratio > 1 (Figure 4). Notably, within the overrepresented terms, six were directly involved in immune system regulation contributing to major immune processes such

as inflammatory response, cytokine regulation, and T cell mobilization corresponding to 34 genes.

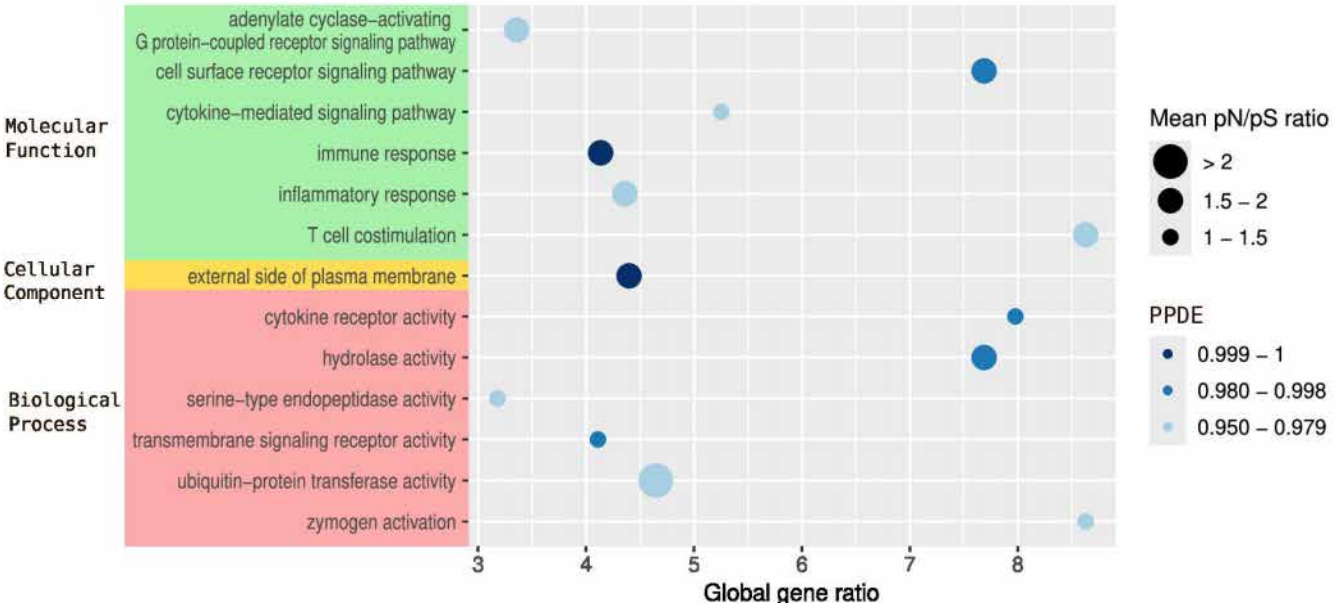

Figure 4. Gene Ontology (GO) enrichment results of genes displaying a pN/pS ratio > 1. GO terms are sorted according to their category (molecular function, cellular component, or biological process). For a given GO term the global gene ratio is the ratio of the proportion of genes displaying the GO term within the targeted group of genes with the proportion of genes displaying the GO term within the total number of protein-coding genes within the genome. The size of the circles varies according to the mean pN/pS ratio of all genes tagged within a GO term. The color of the circles varies according to the value of 1—*p*-value.

4. Discussion

Our study represents an important step in refining the genomic resources available for *P. parva*, a model organism for studying invasive species and host–parasite dynamics. While the initial plan was to construct a de novo genome assembly combining Oxford Nanopore Technologies (ONT) and Illumina sequencing, the low quality of the ONT sequencing data (18,903 contigs with a minimal length of 1000 bases for the more contiguous assembly) necessitated a shift in approach. By focusing on a reference-based assembly strategy using Illumina data, we achieved a highly accurate and comprehensive genomic representation with broad coverage and substantial depth.

The discovery of over 8 million SNPs, equivalent to 7331 SNP/Mbps, between the genome of an invasive *P. parva* individual and a German reference genome reveals striking genomic divergence between the two specimens. This extensive variation strongly suggests that these invasions stem from distinct source populations and invasion trajectories. Such levels of genomic differentiation are unexpected under classical invasion models, which often predict reduced genetic diversity following founder effects and demographic bottlenecks. However, multiple evolutionary mechanisms can account for the retention, and even generation, of genetic variation in invasive populations: First, genetic drift can profoundly shape the genetic landscape of introduced populations. While typically associated with a loss of diversity, drift can also lead to pronounced population structuring [47], even over fine spatial scales, as demonstrated in *Procambarus clarkii* populations introduced to France [48]. This process may reflect serial introductions or founder events from genetically distinct sources. Second, standing genetic variation provides a reservoir of alleles that can rapidly shift in frequency under novel selective pressures [49,50]. This mechanism

facilitates swift adaptation, even in recently founded populations. A notable example is *Apis cerana*, which exhibited marked genomic responses to selection despite a recent and severe bottleneck following its introduction to Australia [51]. Third, de novo mutations, though arising more gradually, can contribute new adaptive variants in populations facing strong selective regimes [52,53]. These mutations may be particularly relevant in cases where standing variation is limited or selection pressures are intense and directional. Together, these mechanisms likely interact to produce the high SNP density and functional differentiation observed between the French and German *P. parva* specimens. These findings underscore the complex evolutionary dynamics that shape invasive genomes and highlight the importance of incorporating genomic data into invasion biology frameworks. Beyond tracing invasion routes, such analyses offer critical insights into how evolutionary forces, from drift to adaptation, enable species to overcome the demographic and ecological constraints of colonization.

Invasive species often succeed in establishing viable populations despite experiencing demographic bottlenecks that are expected to reduce genetic diversity and adaptive potential. The French *P. parva* population is hypothesized to have undergone such a bottleneck [30,34], yet its invasion success raises key questions about the evolutionary mechanisms enabling adaptation in novel environments. One potential explanation lies in the retention or rapid evolution of genetic diversity at loci under selection. High diversity at adaptive loci may be maintained through balancing selection, which preserves multiple alleles within populations despite reduced genome-wide diversity [54,55]. Alternatively, positive selection can drive rapid shifts in allele frequency, either locally at genes associated with specific traits, such as social behavior in *Solenopsis* fire ants [56], or globally across the genome, as observed in *Apis mellifera* under intense environmental selection [57]. Such adaptive signatures have been linked to traits essential for survival in new habitats, including thermal tolerance [58], salinity resistance [54,59], and oxidative stress response [60]. Among the function systems under selection, the immune system occupies a central role in shaping invasion trajectories. The Enemy Release Hypothesis (ERH) posits that invasive species benefit from an ecological reprieve upon escaping their co-evolved pathogens, enabling a reallocation of resources away from immune defense toward other fitness-enhancing traits [61,62]. However, empirical evidence increasingly challenges this view. Several studies have reported elevated expression of immune-related genes [63,64] or increased immune markers [65] in invasive populations relative to their native counterparts. These findings suggest that invasive species, rather than being released from pathogen pressure, may instead encounter novel immunological challenges in the invaded range. As immunologically naive hosts, non-native individuals may experience intense selective pressure from new parasites or pathogens, driving adaptive evolution in immune pathways [66].

During this study we exploited pN/pS ratios to look for signatures of selection within a French *P. parva* genome. However, our results should be interpreted with caution, as the limited sampling depth prevented us from assessing the potential influence of genetic drift, gene flow, or isolation by distance which may affect the pN/pS ratio and obscure signals of selection. Hence, the genes harvesting a pN/pS ratio > 1 are hypothesized to undergo positive selection and only a study led at a larger scale will be able to ascertain these hypotheses.

Intriguingly, our analysis of the *P. parva* genome from an invasive French specimen revealed a pN/pS ratio > 1, which could be interpreted as a signature of positive selection, across multiple genes associated with immune function, suggesting an adaptive response of the immune system to novel environmental challenges. Notably, two genes under potential positive selection, MR1 and H2-Eb1, are integral to the Major Histocompatibility Complex

(MHC), a highly polymorphic gene family pivotal in adaptive immunity through its role in antigen presentation and T cell activation [67,68]. The MHC is known for its rapid evolutionary responsiveness to pathogenic environments; for instance, allele frequency shifts specific to local parasite pressures have been observed to occur within a single generation in three-spined sticklebacks exposed to novel parasites [69]. Similarly, invasive populations of *Rana catesbeiana* maintained native-like MHC diversity despite displaying a marked reduction in mitochondrial cytochrome b diversity, indicating selection-driven conservation of immune function [55]. Beyond the MHC, our findings parallel observations in other invasive species where balancing and positive selection have acted on innate immune genes. In *Procyon lotor* (raccoon), invasive populations exhibited balancing selection in toll-like receptors and cytokine-associated loci [70], patterns echoed in our study by the detection of selection signatures on TLR4 and seven additional genes involved in cytokine receptor activity and/or cytokine-mediated signaling pathway. Taken together, these findings highlight that both innate (e.g., inflammatory response) and adaptive immune processes (e.g., T cell co-stimulation) are hypothesized to be subjected to selective pressures in the invasive French *P. parva* specimen. We identify selection on genes underpinning general pathogen defense mechanisms, as well as those involved in pathogen-specific responses, including CD44a, implicated in antibacterial immunity [71], and IFNGR1, a key mediator of antiviral signaling [72]. This pattern suggests that contrary to the expectations of the ERH, invasive *P. parva* populations may undergo rapid immunogenomic adaptation in response to novel pathogen landscapes. Our study might contribute to a growing body of evidence indicating that immune-related loci constitute critical axes of evolutionary response during biological invasions, shaping not only individual fitness but also broader population-level outcomes.

The postulated immunogenomic plasticity in *P. parva* complements a broader suite of adaptive traits that underpin its ecological success across a wide range of environments. This invasive fish exhibits remarkable tolerance to a variety of abiotic stressors, including temperature fluctuations [19], pollutant exposure [73], and dietary variation [74], each contributing to its capacity to colonize diverse habitats. Within its invasive range, *P. parva* encounters a novel and diverse parasite fauna absent from its native distribution, necessitating an immune system capable of rapid adjustment to a new spectrum of pathogenic threats [21]. These newly encountered parasites include generalist taxa spanning multiple biological kingdoms, such as fungi (e.g., *Blastocladiaceae* and *Chytridiaceae*), oomycetes (e.g., *Saprolegniaceae*), and metazoans (e.g., *Neoechinorhynchidae*) [75,76]. Such pathogen diversity is expected to exert heterogeneous and dynamic selective pressures on the host immune system, thereby favoring the maintenance (or even the enhancement) of immune plasticity in invasive populations. Notably, *P. parva* also serves as an asymptomatic carrier for two notable pathogens: *Sphaerothecum destruens*, a eukaryotic intracellular parasite likely co-introduced with its host following a long co-evolutionary history [28], and fry rhabdovirus, which is absent from the species' native range but present in apparently healthy individuals in invaded regions [77]. The ability to harbor such pathogens without overt clinical signs implies preserved or context-specific immunocompetence, suggesting a flexible immune architecture that may be advantageous even in the species' native context. The genomic divergence we observed in immune-related genes between the French and German individuals of *P. parva* may reflect the interplay of two evolutionary mechanisms. First, differential invasion histories could have led to distinct demographic bottlenecks, with varying intensities of genetic drift causing lineage-specific retention or erosion of immune diversity. Second, geographically distinct parasite communities in each invaded region may have driven local adaptation via selection on pre-existing alleles or de novo mutations, shaping divergent immunogenomic profiles between populations. Together, these findings reinforce the concept that immune system plasticity and evolvability are

pivotal to invasion success. They also underscore the importance of pathogen-mediated selection as a central force in shaping the evolutionary trajectory of invasive vertebrates facing novel biotic environments.

5. Conclusions

This study provides the first comprehensive genome-wide comparison of *P. parva* genetic diversity across two invasive specimens, offering critical insights into the evolutionary dynamics underlying its colonization of Europe. The identification of over eight million SNPs between individuals sampled from France (this study) and Germany (using the whole genome available from a previous study) underscores a marked genomic divergence, consistent with distinct invasion histories and source populations. These findings reinforce the view that parallel invasions can follow independent demographic and adaptive trajectories, even within the same species. Our analysis highlights the central role of immune-related genes in the adaptation of *P. parva* to novel environments. Signals of positive and diversifying selection across a wide array of immune functions, including both innate mechanisms (e.g., inflammatory response) and adaptive pathways (e.g., T cell co-stimulation), point to immune plasticity as a key enabler of invasion success. This immunogenomic flexibility likely contributes to the species' remarkable tolerance to diverse environmental stressors and its capacity to persist in pathogen-rich ecosystems. From a theoretical standpoint, our results support emerging frameworks that emphasize functional genetic diversity, rather than overall genetic richness alone, as a primary driver of invasion dynamics. This is consistent with comparative work in other systems, such as the contrasting invasion outcomes of Round goby and Tubenose goby, where functional divergence in thermal tolerance genes explained differential success [78]. Looking forward, expanding the scope of immunogenomic surveys across multiple invasive populations of *P. parva* could illuminate the extent of local adaptation to native parasite communities and help predict invasion potential in newly colonized regions. Such work could also identify the genomic predictors of resistance to co-invasive pathogens like S. destruens, offering actionable targets for biocontrol.

Ultimately, a deeper understanding of the genomic architecture of immune function may pave the way for novel mitigation strategies. For instance, gene-editing approaches targeting host resistance loci, such as CRISPR-mediated knockouts, could, in principle, disrupt the symbiotic persistence of *P. parva* and its associated pathogens. While still speculative, such strategies highlight the transformative potential of functional genomics for the management of biological invasions and the protection of native biodiversity.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fishes10060297/s1, Table S1: bcftools commands used for variant filtering in the VCF file. Each step aims to remove low-confidence or potentially biased variant calls.

Author Contributions: Conceptualization, M.C. and R.E.G.; formal analysis, T.D.; investigation, M.C., T.D., J.G., and R.E.G.; writing—original draft preparation, M.C. and T.D.; writing—review and editing, M.C. and R.E.G.; funding acquisition, M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Agence Nationale de la Recherche (ANR-22-CE02-0013-01 DRIVE).

Institutional Review Board Statement: Our study did not require approval as the sampling and euthanasia of the specimen were conducted by "La Fédération de l'Indre pour la Pêche et la Protection du Milieu Aquatique (FDAAPPMA36)" which operates under its own authorizations granted by the french government. Moreover, the ANR Drive project, under which the study was conducted, received the Nagoya agreement N°TREL2302365S/661 from the "Ministère de la Transition Écologique

et de la Cohésion des Territoires" as well as an internationally recognized certificate of compliance constituted from information on the permit or its equivalent made available to the Access and Benefit-sharing Clearing-house N°ABSCH-IRCC-FR-263712-1, allowing us to realize the dissection of the specimen on the field.

Informed Consent Statement: Not applicable.

Data Availability Statement: This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JBOLDD000000000. The version described in this paper is version JBOLDD010000000.

Acknowledgments: We acknowledge "La Fédération de l'Indre pour la Pêche et la Protection du Milieu Aquatique (FDAAPPMA36)" and notably Bruno Barbey for their support in the fish collection.

Conflicts of Interest: The authors declare no conflicts of interest.

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