

River dams restrict the upstream spread of non-native freshwater shrimps and provide refugia for native shrimps

Yusuke Fuke

yfa73986@gmail.com

Setsunan University: Setsunan Daigaku <https://orcid.org/0000-0001-6404-6241>

Ryosuke Ishii

Kagawa University: Kagawa Daigaku

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Abstract

Dams negatively affect freshwater organisms by fragmenting habitats, but they may also protect native populations by restricting the upstream dispersal of non-native species. However, evidence for this potential conservation function remains limited for freshwater invertebrates. We investigated whether conventional dams and check dams restrict the upstream dispersal of non-native *Neocaridina* shrimps in five river systems on Shodoshima Island, Japan. Because the native *N. denticulata* and non-native *N. davidi* are morphologically similar and can hybridize, we analyzed mitochondrial DNA and genome-wide SNPs to identify the species and compare their genetic composition in six replicated upstream–downstream population pairs across dams. Both analyses consistently indicated that the native species occurred primarily upstream of dams, whereas the non-native species occurred primarily downstream. In several rivers, genetic signatures of interspecific hybridization were detected downstream of dams, along with mtDNA introgression and the loss of genomic ancestry derived from one of the parental species. The occurrence of the non-native species in downstream reaches is consistent with its local availability and use as bait for marine recreational fishing, suggesting that these populations may have originated from bait shrimp that were released or escaped into the wild. Our findings indicate that dams may restrict the upstream spread of non-native shrimps and highlight the need to prevent their further range expansion when implementing river-connectivity restoration measures, such as the installation of fishways or bypass channels and dam removal.

Introduction

Freshwater ecosystems, which are rich in biodiversity, are exposed to various anthropogenic stresses, such as river development and biological invasions. A recent global assessment of freshwater fishes, decapods, and odonates estimated that approximately one-quarter of these species are threatened with extinction, with the proportion reaching approximately 30% among decapods (Sayer et al. 2025). However, because species' responses to environmental change vary according to their ecological traits and trophic positions (Betts et al. 2014; Thackeray et al. 2016), findings derived from a limited range of taxonomic groups may not adequately represent the effects of environmental change on freshwater biodiversity as a whole (Sayer et al. 2025). It is therefore necessary to evaluate how potential factors of biodiversity loss affect species and local populations in relatively understudied taxonomic groups, including freshwater decapods.

Dams generally have negative effects on native freshwater organisms because they reduce river connectivity, impede migration and gene flow among populations, and alter flow regimes, water temperature, sediment transport, and habitat conditions (Reid et al. 2019; Wu et al. 2019). In particular, for diadromous fishes that migrate between rivers and the ocean, dam-induced fragmentation of migration routes negatively affects community composition, species diversity, population size, genetic diversity, and population genetic structure (Chan et al. 2025). On the other hand, dams can also restrict the upstream movement of non-native species and may allow habitats above these barriers to serve as refugia for native populations (McLaughlin et al. 2013). Although these upstream populations may still

be negatively affected by dams, they may be protected from impacts associated with non-native species, including predation, hybridization, and the transmission of diseases and parasites (Havel et al. 2015; Prati et al. 2024). Thus, the conservation consequences of habitat fragmentation caused by dams are not necessarily uniform. However, most existing knowledge is limited to fishes, highlighting the need to accumulate evidence from other taxonomic groups.

A recent study in the Yumesaki River system, Hyogo Prefecture, Japan, reported that populations consisting exclusively of the native freshwater shrimp *N. denticulata* persisted upstream of the dam, whereas the native species had been replaced by the non-native *N. davidi* at most sites downstream of the dam (Ishii & Fuke 2025). The genus *Neocaridina* comprises landlocked freshwater shrimps that complete their entire life cycle in freshwater (Cai 1996) and can therefore maintain populations in habitats isolated by dams. These findings suggest that dams can restrict the upstream dispersal of non-native shrimps and thereby act as barriers that protect native populations (Ishii & Fuke 2025). However, because these findings are based on a case study from a single river system, the generality of this pattern remains unclear. Addressing this knowledge gap is important because conservation measures designed to restore river connectivity, such as dam removal and the installation of fishways or bypass channels (Foley et al. 2017; Silva et al. 2018), may inadvertently contribute to the spread of invasive non-native species.

To assess the generality of the role of dams in restricting the dispersal of non-native freshwater shrimps, we compared the genetic structure of *Neocaridina* shrimps collected upstream and downstream of conventional dams and check dams in multiple rivers on Shodoshima Island in the Seto Inland Sea, Japan. Non-native *Neocaridina* shrimps are traded in Japan as live bait and ornamental pets (Niwa 2010). Recreational fishing is popular on Shodoshima Island, where exotic freshwater shrimps are commercially available as fishing bait (Imai et al. 2020). The likelihood that bait shrimp are released or escape into the wild is therefore expected to be relatively high in coastal areas and near river mouths, where much of the fishing activity occurs. We predicted that, if opportunities for the introduction of non-native shrimps are concentrated in downstream reaches and their subsequent upstream dispersal is restricted by dams, non-native species would be detected primarily downstream of dams, whereas native species would persist upstream. These *Neocaridina* species are well suited for testing this hypothesis because their broad environmental tolerance allows them to inhabit a variety of habitats from the upper to lower reaches of rivers (Niwa & Yokoyama 1993; Ishii & Fuke 2025), thereby limiting the extent to which environmental conditions determine their distributions. Because native and non-native *Neocaridina* species are morphologically similar and can hybridize (Onuki & Fuke 2022), we used a combination of mitochondrial DNA (mtDNA) and genome-wide polymorphism analyses to identify species and characterize the genetic composition of *Neocaridina* populations upstream and downstream of dams. Although the taxonomic assignments of the lineages corresponding to the native *N. denticulata* and the non-native *N. davidi* remain disputed (Shih et al. 2024; Fuke 2024), we followed the identifications proposed by Onuki and Fuke (2022), which were based on quantitative morphological analyses and genome-wide SNP data.

Materials and methods

Field survey

Sampling of *Neocaridina* shrimps was conducted on Shodoshima Island, Kagawa Prefecture, Japan, from 25 to 27 September 2024. Shodoshima Island, located in the Seto Inland Sea, has an area of 153 km² and contains several dammed rivers. Fourteen sampling sites were selected upstream and downstream of six dams, including conventional dams and check dams, across five river systems (Fig. 1, Table 1, S1, S2). Prior to sampling, a special catch permit was obtained from Kagawa Prefecture (Permit No. 14). Specimens were collected using hand nets, and at least eight individuals were obtained from each site. The specimens were preserved in 100% ethanol in the field, and after DNA extraction, they were transferred to 70% ethanol. All specimens were catalogued and deposited in the Zoological Collection of Kyoto University, Japan (KUZ Z6588–Z6605, Z6607–Z6609; Table S3).

Table 1
Information on sampling sites on Shodoshima Island

Site ID	Name	River System	Date	Specimen ID	Species
A1	Sangoro-Reservoir	Yasuda-Oo	2024-09-25–26	KUZ Z6588–Z6589	<i>Neocaridina denticulata</i>
A2	Yasuda-Oo River (lower)	Yasuda-Oo	2024-09-25	KUZ Z6590	<i>Neocaridina denticulata</i>
B1	Betto River (upper)	Betto	2024-09-26	KUZ Z6591	<i>Neocaridina</i> spp. (hybrid zone)
B2	Betto River (lower)	Betto	2024-09-26	KUZ Z6592	<i>Neocaridina davidi</i>
DD1	Kaerugo Reservoir	Denpo	2024-09-26	KUZ Z6593	<i>Neocaridina denticulata</i>
DD2	Denpo River (middle)	Denpo	2024-09-26	KUZ Z6594	<i>Neocaridina</i> spp. (hybrid zone)
DD3	Denpo River (lower)	Denpo	2024-09-26	KUZ Z6595	<i>Neocaridina davidi</i>
DT1	Nakayama Reservoir	Denpo (Tonogawa river tributary)	2024-09-26	KUZ Z6596	<i>Neocaridina denticulata</i>
DT2	Shin-Nakayama Reservoir	Denpo (Tonogawa river tributary)	2024-09-26	KUZ Z6597	<i>Neocaridina denticulata</i>
DT3	Tonogawa River (lower)	Denpo (Tonogawa river tributary)	2024-09-26	KUZ Z6598	<i>Neocaridina davidi</i>
K1	Katsura River (upper)	Katsura	2024-09-27	KUZ Z6599	<i>Neocaridina denticulata</i>
K2	Katsura River (lower)	Katsura	2024-09-27	KUZ Z6600	<i>Neocaridina davidi</i>
Y1	Yoshida River (upper)	Yoshida	2024-09-25	KUZ Z6601	<i>Neocaridina denticulata</i>
Y2	Yoshida River (lower)	Yoshida	2024-09-25	KUZ Z6602	<i>Neocaridina denticulata</i>
Supplementary Information					

To investigate the local trade in *Neocaridina* shrimps, we interviewed staff at three fishing-tackle shops (hereinafter referred to as shops A, B, and C) on Shodoshima Island regarding the sales history, suppliers, and intended uses of the shrimp sold under the Japanese commercial name “Butsu-ebi.”

DNA extraction and mtDNA sequencing

Eight individuals per site were used for DNA analysis, for a total of 112 individuals. Total DNA was extracted from the fifth pereopod or pleopod using the Monarch Genomic DNA Purification Kit (New England Biolabs). A fragment of the mitochondrial COI region was amplified by PCR using primers LCO-nc (5'-TTG TCA ACA AAC CAC AAA GAY ATT GG-3') and HCO-nc (5'-ATA CYT CAG GGT GYC CAA AGA ATC A-3') (Onuki & Fuke 2022), in a 12.5-μL reaction mixture containing 0.25 μL of PrimeSTAR GXL DNA polymerase (Takara Bio), 2.5 μL of 5× PrimeSTAR GXL buffer, 1.0 μL of dNTP mixture, 0.75 μL of each 5 μM primer, 0.8 μL of template DNA, and 6.45 μL of ultrapure water. The PCR amplification conditions were as follows: initial denaturation at 98°C for 30 s; 30 cycles of denaturation at 98°C for 10 s, annealing at 55°C for 15 s, and extension at 68°C for 45 s; and a final extension at 68°C for 30 s. The PCR products were purified using ExoSAP-IT Express (Applied Biosystems) and sequenced from both the 5' and 3' ends. Sanger sequencing was outsourced to Eurofins Genomics (Tokyo, Japan). The resulting sequences were inspected and edited to correct base-calling errors using Unipro UGENE 52.0 (Okonechnikov et al. 2012). All sequences (653 bp) were deposited in the International Nucleotide Sequence Database Collaboration (INSDC) via the DNA Data Bank of Japan (DDBJ; accession numbers LC942877–LC942988; Table S3).

mtDNA analyses

To identify the COI haplotypes of *Neocaridina* shrimps from Shodoshima Island and compare them with those reported in previous studies, haplotype detection was performed using VSEARCH 2.31.0 (Rognes et al. 2016) from a dataset comprising the COI sequences obtained in this study and additional *Neocaridina* COI sequences retrieved from INSDC (accessed on 21 July 2026) and from Cui et al. (2023). Sequence regions extending beyond the 653-bp region determined in this study were trimmed to that region, whereas shorter sequences were retained at their original lengths. Sequences that were identical across their overlapping regions were operationally treated as the same haplotype.

Because novel haplotypes were detected, a maximum-likelihood phylogenetic tree was inferred using IQ-TREE 2.2.2.6 (Minh et al. 2020) to determine their phylogenetic positions. Phylogenetic analyses were conducted separately for two datasets: dataset 1, comprising haplotypes detected among individuals collected from Shodoshima Island, with *N. spinosa* (AB300188) included as the outgroup; and dataset 2, comprising dataset 1 supplemented with all haplotypes retrieved from the INSDC. The best-fit nucleotide substitution model for each dataset was selected using ModelFinder (Kalyaanamoorthy et al. 2017) based on the Bayesian information criterion (BIC). The HKY + F + R2 and TN + F + I + R3 models were selected for datasets 1 and 2, respectively. Branch support was assessed using the ultrafast bootstrap method with 1,000 replicates (Hoang et al. 2018). The resulting phylogenetic trees were visualized using FigTree v1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>). Species and clade names were assigned to the lineages recovered in the phylogenetic analyses following Onuki and Fuke (2022) and Fuke (2024).

MIG-seq library preparation

We performed multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq; Suyama & Matsuki 2015) to estimate the population structure based on genome-wide single nucleotide polymorphisms (SNPs). We prepared the MIG-seq library according to the methods described by Suyama et al. (2022) and Watanabe et al. (2020). Sequencing of the pooled library was outsourced to Rhelixa Co., Ltd. (Tokyo, Japan) and performed on an Illumina NovaSeq X Plus platform. The raw data were deposited in the DDBJ Sequence Read Archive (DRA) under BioProject accession number PRJDB42801.

Genome-wide SNP analyses

Quality control and trimming of the raw MIG-seq reads were performed using fastp 0.20.1 (Chen et al. 2018). The first 17 bases were removed from Reads 1 and 2 ($F = 17$, $f = 17$), and reads containing low-quality bases ($< Q30$) or adapter sequences were discarded ($q = 30$). The remaining reads were then trimmed to a final length of 130 bases ($B = 130$, $b = 130$, $l = 130$).

We performed SNP calling using the `denovo_map.pl` pipeline in Stacks 2.68 (Rochette et al. 2019) with the following settings: minimum depth (5; $m = 5$), maximum genetic distance allowed between stacks (5; $M = 5$), minimum percentage of individuals in which a locus was present (50%; $R = 0.5$), minimum minor allele count (2; $\text{min-mac} = 2$), maximum observed heterozygosity (75%; $\text{max-obs-het} = 0.75$), and only the first SNP per locus (`write-single-snp`). Downstream analyses were conducted on the files generated in PLINK format.

Principal component and clustering analyses were performed to estimate genetic population structure. Principal component analysis (PCA) was performed using PLINK v1.90b5 (Purcell et al. 2007). Clustering analysis was performed using ADMIXTURE 1.3.0 (Alexander et al. 2009) for $K = 1-10$, with 100 runs at different seeds for each value of K . The results were summarized based on cross-validation (CV) errors. For each value of K , we reran the analysis using the seed value that showed the lowest CV error to obtain the final output. These results were summarized and visualized in R 4.3.1 (R Core Team 2023).

Results

Interviews at fishing-tackle shops

All three fishing-tackle shops had previously sold *Neocaridina* shrimps (Butsu-ebi) as groundbait for rockfish (*Sebastes* spp.) fishing. Shop staff also noted that demand for these shrimps as fishing bait had declined because of decreases in the abundance of *Sebastes* spp. around Shodoshima Island. Shop A had obtained shrimps of unknown origin, whereas Shop C had obtained shrimps imported from China. Both shops discontinued sales between 2019 and 2022 after their suppliers stopped distributing the shrimps. In contrast, Shop B continues to sell locally sourced shrimps from Shodoshima Island during winter. A staff member at Shop C also reported that, more than 30 years ago, anglers commonly brought live *Neocaridina* shrimps from Honshu to Shodoshima Island for use as fishing bait. Because these

shrimps were not available for sale at the time of our survey, we were unable to determine which species were being traded.

mtDNA analysis

A total of 312 haplotypes were identified from 1,448 mitochondrial COI sequences obtained in this study and previous studies (Fig. S1). Among the 112 individuals collected from 14 sites across five river systems on Shodoshima Island, 25 haplotypes were detected and assigned to *Neocaridina denticulata* (native), *N. davidi* (non-native), or *N. koreana* (non-native), of which 12 were novel haplotypes (Fig. 2A). Eleven haplotypes were assigned to *N. denticulata*. These haplotypes tended to occur upstream of dams, although they were also detected downstream of dams in the Denpo River system (DD2, DD3, and DT3), the Yasuda-Oo River system (A2), and the Yoshida River system (Y2). *Neocaridina davidi* comprises three clades (Onuki & Fuke 2022), of which two haplotypes belonging to Clade A and 11 belonging to Clade B were detected on the island. These haplotypes tended to occur downstream of dams and were detected upstream of a dam only in the Betto River system (B1). Haplotypes of both *N. denticulata* and *N. davidi* were detected at five sites (B1, DD2, DD3, DT3, and Y2). A single haplotype assigned to *N. koreana* was detected in one individual at each of two sites upstream of dams (DT1 and DT2). Sequences belonging to the same clade as this haplotype have been obtained from Korea and Japan (Fig. S1). However, because none of these sequences were associated with identifications based on morphological characters (Fujita et al. 2011; Shih et al. 2017; Nagai & Imai 2024; Park et al. 2019), the assignment of this haplotype to *N. koreana* should be regarded as provisional.

Genome-wide SNP analysis

Principal component analysis (PCA) was performed using 337 SNP loci, each with a missing-data rate of < 50%, obtained from 112 individuals across 14 populations. The *Neocaridina* shrimps from Shodoshima Island formed two distinct clusters along the first principal component (PC1), which explained 21.4% of the total variance. These clusters largely corresponded to populations located upstream and downstream of dams, respectively (Fig. 2B). Some individuals from the middle reach of the Denpo River (DD2) and the downstream reaches of the Betto (B2) and Tonogawa (DT3) rivers were positioned between the two clusters. Along the second principal component (PC2), the population from the upper reach of the Yasuda-Oo River (A1) was separated from all other populations.

ADMIXTURE analysis based on the same dataset yielded the lowest mean and minimum CV errors at $K = 3$. The ancestry patterns inferred at $K = 3$ were consistent with the PCA results. Individuals positioned between the two clusters along PC1 showed mixed ancestry comprising the two corresponding ancestry components in the ADMIXTURE analysis (Fig. 2C). The ancestry component occurring predominantly upstream of dams broadly corresponded to the mitochondrial lineage of *N. denticulata*. Despite showing no mitochondrial DNA differentiation from the other populations, the population from the upper reach of the Yasuda-Oo River (A1) was predominantly composed of a distinct nuclear ancestry component, which was also partially represented in the population from Kaerugo Reservoir (DD1). The ancestry component

occurring predominantly downstream of dams broadly corresponded to the mitochondrial lineage of *N. davidi*. Evidence of interspecific hybridization was detected in populations B2, DD2, DD3, DT1–DT3, K2, and Y2, based on either mixed nuclear ancestry or discordance between mitochondrial haplotypes and nuclear ancestry. At DD3, an individual carrying an *N. denticulata* mitochondrial haplotype showed little detectable *N. denticulata* ancestry in its nuclear genome. Conversely, individuals from DT1 and DT2 carrying an *N. koreana* mitochondrial haplotype, as well as individuals from Y2 carrying an *N. davidi* mitochondrial haplotype, exhibited exclusively *N. denticulata*-associated nuclear ancestry.

Discussion

Effects of dams on *Neocaridina* shrimps

This study demonstrated that, across multiple river systems, riverine structures that restrict the movement of non-native species can act as barriers that protect native species. Although only one previous case study has documented this pattern in *Neocaridina* shrimps (Ishii & Fuke 2025), the upstream reaches of all six dams examined on Shodoshima Island served as the primary habitats of the native species. Two conditions may be necessary for dams to provide such protection: the absence of direct introductions of non-native species into upstream reaches and the landlocked life history of the native species. From the perspective of biodiversity conservation, river connectivity is commonly restored by installing fishways or bypass channels or, where feasible, by removing dams (Foley et al. 2017; Silva et al. 2018). Because no such connectivity-restoration measures were implemented at our study sites, the dams may have limited the upstream range expansion of non-native species. Our findings highlight the need to implement measures to prevent the range expansion of non-native shrimps when undertaking such conservation actions.

The only site at which the non-native species occurred upstream of a dam was B1, upstream of Uchinomi Dam. This dam was completed relatively recently, in 2013, and the observed distribution may therefore reflect the establishment of the non-native species upstream of the present dam before its construction. This explanation is consistent with the fact that the spread of non-native *Neocaridina* species in Japan began to be reported in the 2000s (Nishino 2017). Alternatively, because no evidence of interspecific hybridization was detected at B1 in either the mitochondrial or nuclear DNA data, the population may have originated from a recent accidental release. Uchinomi Dam is 43 m high, making it the third-highest dam on Shodoshima Island (Table S1); therefore, direct upstream movement over the dam itself is highly unlikely. Regardless of the underlying mechanism, non-native species were not detected upstream of any other dam examined in this study, indicating that their occurrence at B1 represents a rare case.

Discordance between mitochondrial DNA and the nuclear genome was observed in the Sangoro Reservoir population (A1). Although the mitochondrial lineage showed no clear differentiation from those in downstream populations, genome-wide SNP data distinguished the A1 population from the other *N. denticulata* populations. Sangoro Reservoir, which was constructed in 1924, is located upstream

of Awaji Dam, which was completed in 1980. The construction of the reservoir and the dam may therefore have isolated the upstream population and reduced its effective population size, allowing genetic drift to alter the frequencies of pre-existing nuclear genetic variants. Testing this scenario will require additional sampling of riverine populations around A1 and larger sample sizes at each site.

Patterns of interspecific hybridization

The combined analysis of mitochondrial DNA and genome-wide SNPs revealed that interactions between native and non-native species can lead to different genetic consequences. Hybridization between native *N. denticulata* and non-native *N. davidi* has been reported (Onuki & Fuke 2022), as has the replacement of native species by non-native species (Ishii & Fuke 2025). In the present study, several populations, including B2, DD3, and DT3, appeared to represent cases in which the native species had been replaced by the non-native species through hybridization. Interestingly, we also identified a contrasting pattern at Y2. Although mitochondrial introgression indicated a past introduction of the non-native species into the native population, no detectable trace of non-native ancestry remained in the nuclear genome. This finding suggests that, under certain conditions, native populations may resist genetic replacement by non-native species.

The direction of such replacement is likely influenced by various physical and biological factors, including environmental conditions within rivers and the relative abundances of the two species. However, these factors could not be evaluated using the data obtained in this study. Further research is therefore needed to elucidate the ecological mechanisms that determine whether native species are replaced by non-native species or whether non-native species are excluded, as understanding these mechanisms is crucial for conserving native freshwater ecosystems.

Origin and range expansion of the non-native species

The non-native species *N. davidi* has become established in many parts of the world (Prati et al. 2025) and has caused irreversible impacts on native species (Katayama et al. 2017; Onuki & Fuke 2022; Ishii & Fuke 2025). Therefore, elucidating the processes underlying its range expansion is an urgent priority. Our results revealed the occurrence of *N. davidi* in the downstream reaches of multiple river systems. Furthermore, at the sites located downstream of the dam in the Denpo River system, the proportion of genetic ancestry derived from *N. davidi* was higher at the downstream site (DD3) than at the upstream site (DD2). This spatial pattern provides evidence that the species is expanding its range from downstream to upstream reaches. *Neocaridina davidi* is commercially available as bait for marine fishing and as an ornamental pet (Niwa 2010; Prati et al. 2025; present study). Individuals used as fishing bait, including unused surplus bait, may be released or escape into coastal and downstream areas (Saito et al. 2011). Indeed, several previous studies have suggested that *N. davidi* has invaded and become established in downstream reaches (Nishida 2016; Katayama et al. 2017), and our results support these findings. Moreover, the inferred upstream range expansion of *N. davidi* is consistent with the upstream migratory behavior reported in the closely related species *N. denticulata* (Niwa & Yokoyama 1997).

The mitochondrial haplotype detected in one individual from each of the adjacent Nakayama Reservoir (DT1) and Shin-Nakayama Reservoir (DT2), and provisionally assigned to the Korean species *N. koreana*, may have resulted from an introduction. Sequences highly similar to this haplotype ($\geq 99\%$ sequence identity) have been reported from southern Korea (MK907783), Kyoto Prefecture (LC830190), Hiroshima Prefecture (LC324777), and Shimane Prefecture (AB524967) (Fujita et al. 2011; Nagai & Imai 2024; Shih et al. 2017; Park et al. 2019). Because no distinct ancestry component was detected in the nuclear genomes of these individuals, the observed pattern may reflect historical introgression involving only the mitochondrial genome.

Neocaridina shrimps were imported from Korea to Japan between 1969 and 2001 (Niwa 2010), and this haplotype may have been introduced into this river system during that period. The restriction of this haplotype to sites upstream of the dam contrasts with the spatial pattern observed in *N. davidi*. However, because this pattern was observed in only one river system, the occurrence of this haplotype upstream of the dam is currently best explained by an accidental or opportunistic release in the past. Analyses based on larger sample sizes, including samples from Korean populations, are required to determine whether the “*N. koreana*” haplotypes recorded in Japan are native or introduced.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

All animal experiments were approved by the Institutional Animal Care and Use Committee of the National Institute of Genetics (approval numbers R5-23 and R6-17).

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Author Contributions

Yusuke Fuke contributed to the conception and design of the study, data analysis, and data curation. Field surveys and molecular experiments were conducted by all authors. Yusuke Fuke wrote the first draft of the manuscript, and all authors reviewed and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

All specimens examined in this study were catalogued and deposited in the Zoological Collection of Kyoto University, Japan (KUZ Z6588–Z6605 and KUZ Z6607–Z6609). All mitochondrial DNA sequences generated in this study were deposited in the International Nucleotide Sequence Database Collaboration (INSDC) under accession numbers LC942877 – LC942988. The raw MIG-seq reads were deposited in the DDBJ Sequence Read Archive (DRA) under BioProject accession number PRJDB42801.

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44. Statements & Declarations

Figures

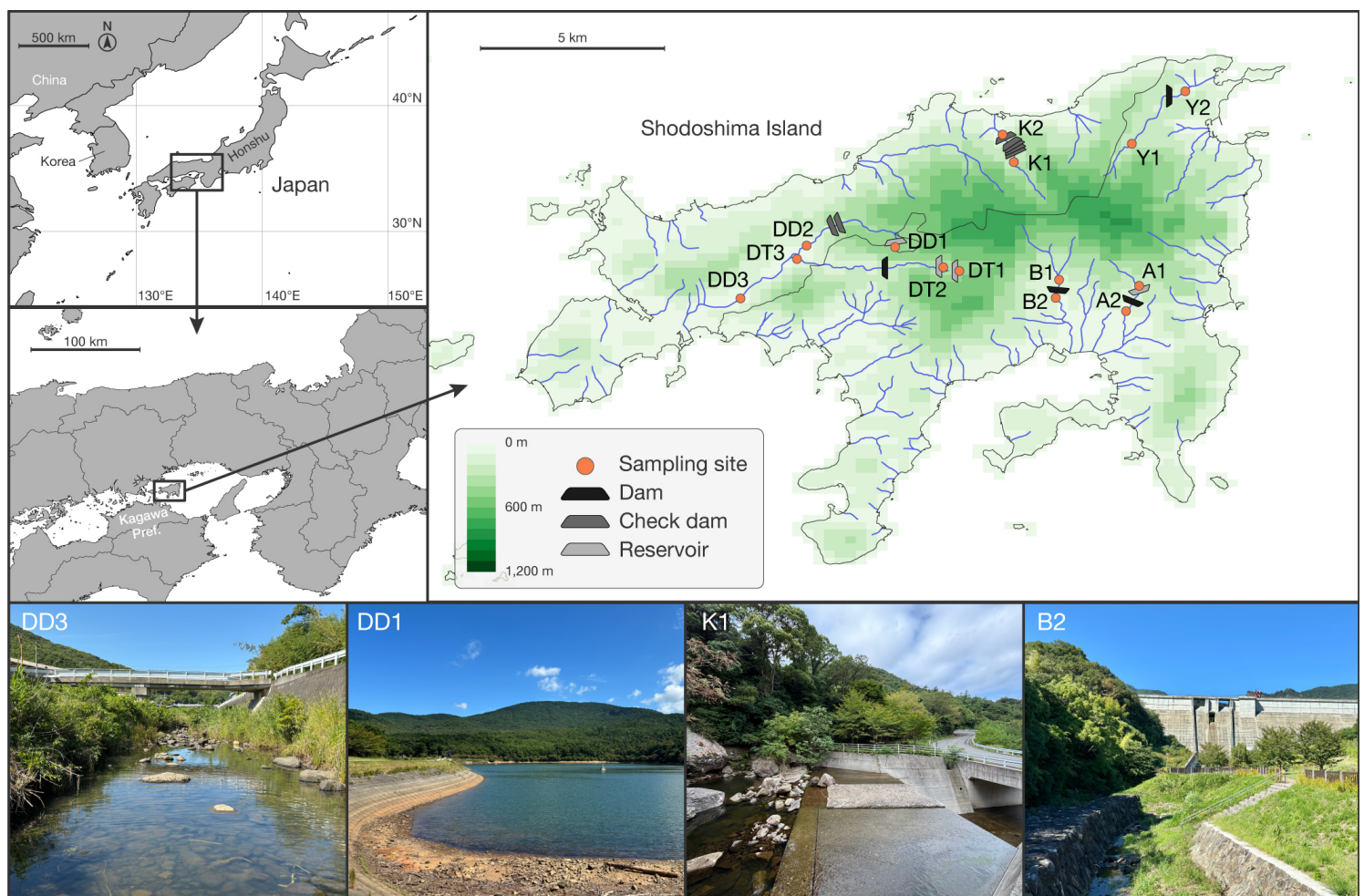


Figure 1

Map showing the location of Shodoshima Island, Kagawa Prefecture, Japan, sampling sites and dams. The four photographs below the map show representative sampling sites: from left to right, a downstream river reach (DD3), a reservoir (DD1), a check dam (K1), and a dam with its associated downstream reach (B2)

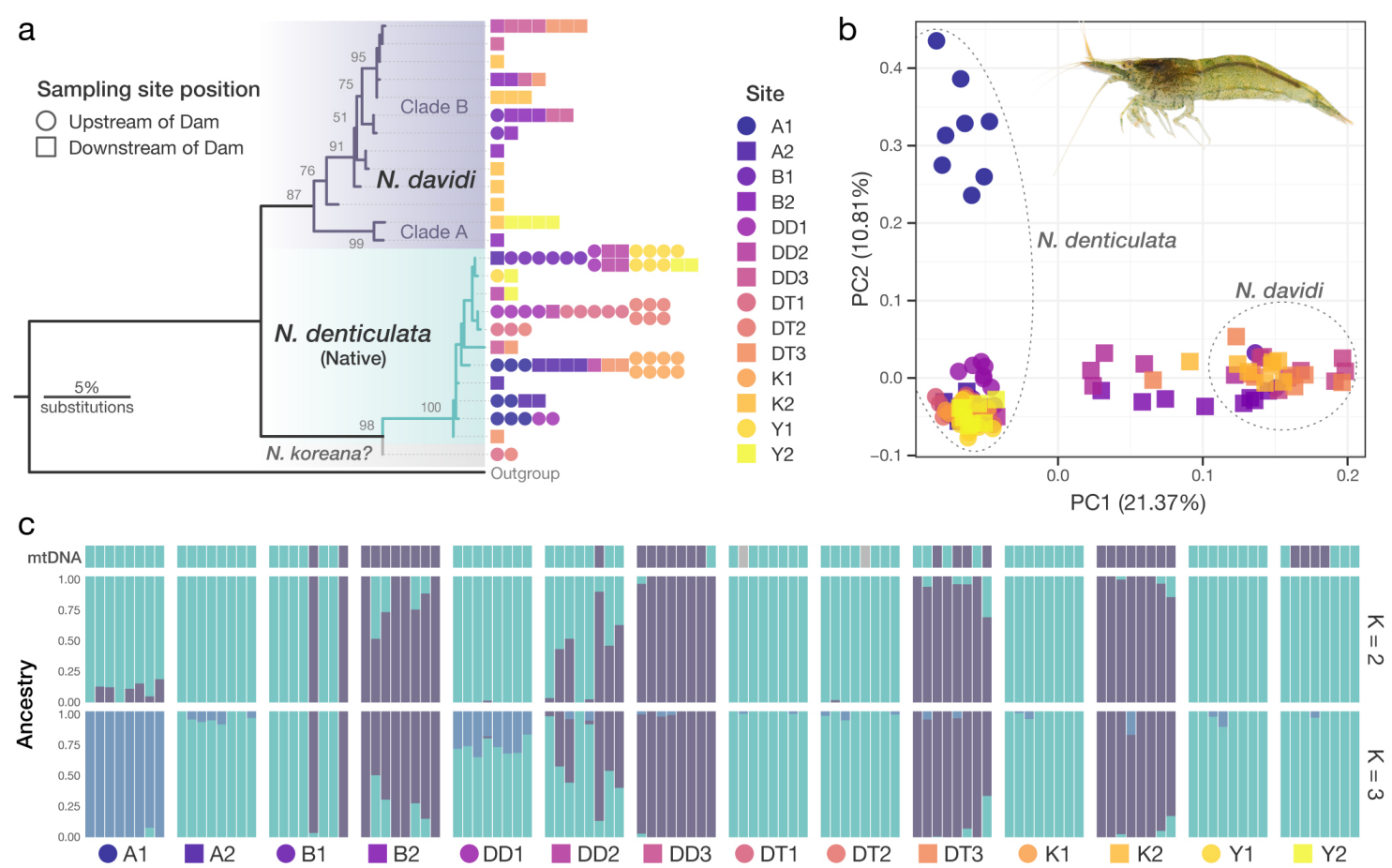


Figure 2

Results of the genetic analyses. **a**, Haplotype-based maximum-likelihood phylogenetic tree inferred from the mitochondrial DNA COI region (653 bp). Numbers at the branches indicate ultrafast bootstrap support values. The shapes of the tip symbols indicate whether the sampling sites were located upstream (circles) or downstream (squares) of dams, and the colors indicate sampling sites. **b**, Principal component analysis (PCA) based on 337 genome-wide SNPs. Symbol shapes and colors correspond to those in panel **a**. **c**, ADMIXTURE analysis at $K = 2$ and 3 based on the same SNP dataset used for the PCA. The squares above the bar plots indicate mtDNA lineages, with colors corresponding to those in panel **a**

Supplementary Files

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