国立科博専報, 54号, pp.291–327, 2026 年 3 月 28 日
Mem. Natl. Mus. Nat. Sci., No. 54, pp.291–327, March 28, 2026

Fishes from the Moats and Ponds of the Imperial Palace Inner Garden, Tokyo, Japan in 2024–2025

Gento Shinohara[1, 2, 3*], Munenori Kishida[4], Masanori Nakae[1, 5], Kyoji Fujiwara[1, 6], Mao Sato[1,7] & Masayoshi Hayashi[4]

[1]Department of Zoology, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba, Ibaraki 305–0005, Japan
[*]E-mail: s-gento@kahaku.go.jp
[2]Hokkaido University Museum,
Nishi 8-chome, Kita 10-jo, Kita-ku, Sapporo, Hokkaido 060–0810, Japan
[3]Tokyo University of Technology,
1404–1 Katakuramachi, Hachioji, Tokyo 192–0982, Japan
[4]Biological Laboratory of Imperial Palace, Emperor Emeritus' Household, Imperial Household Agency
1–1, Chiyoda, Chiyoda-ku, Tokyo 100–8111, Japan
[5]School of Integrative and Global Majors, University of Tsukuba,
1–1–1 Tennodai, Tsukuba, Ibaraki 305–8577, Japan
[6]The Kyoto University Museum
Yoshida honmachi, Sakyo-ku, Kyoto 606–8317, Japan
[7]Faculty of Science, University of the Ryukyus
1 Senbaru, Nishihara, Okinawa 903–0213, Japan

Abstract. During Phase III of the Imperial Palace Biodiversity Survey, fish surveys were conducted in the moats and ponds of the Imperial Palace Inner Garden during the spring and autumn 2024, and summer 2025. This was the first fish survey of the Inner Garden in 25 years, because no fish surveys had been conducted in Phase II following Phase I. Whereas Phase I targeted the Kami-dokan-bori, Naka-dokan-bori and Shimo-dokan-bori moats, Phase III was expanded to include the Hasuike-bori, Inui-bori, Hirakawa-bori, Tenjin-bori and Hakuchobori moats, Ninomaru-ike Pond, and additional small waterbodies within the Fukiage Imperial Garden. Specimens collected during the survey period were deposited at the Tsukuba Research Departments of the National Museum of Nature and Science, Tokyo. In addition to collection surveys and visual observations, environmental DNA analyses were conducted, the overall results confirming the presence of at least 15 species within the Inner Garden. The collection survey also confirmed the expanded distribution of the gobiid *Tridentiger brevipinnis*, previously recorded only in the Outer Garden moats, for the first time in the Inner Garden moats (Hirakawa-bori and Tenjin-bori moats). Species confirmed solely through visual observations were the two cyprinids *Ctenopharyngodon idellus* (Inui-bori and Tenjin-bori moats) and *Hypophthalmichthys molitrix* (Tenjin-bori Moat). Specimens of the anguillid *Anguilla japonica* (Hirakawa-bori and Tenjin-bori moats) and cobitid *Misgurnus chipisaniensis* (Naka-dokan-bori and Shimo-dokan-bori moats), whose distributions were suggested by eDNA, were not collected. The agreement rate between eDNA detection of species and those confirmed by collection surveys and visual observations was relatively low at Naka-dokan-bori Moat (60%), but averaged 88% across all water bodies. At Fukiage Ohike Pond, the cyprinid *Pseudorasbora pumila* had been stocked in the past as an attempt at species conservation. During this survey, its presence was confirmed both by collection and eDNA detection. However, no evidence for the persistence of the adrianichthyid *Oryzias latipes*, initially reported in Naka-dokan-bori Moat between 1986 and 1999, was found. On the other hand, the poecilid *Gambusia affinis*, considered a competitor

of *O. latipes*, was collected in large numbers in many of the waterbodies, including Naka-dokan-bori Moat. The cyprinid *Carassius* sp. "Ginbuna," which had been collected frequently in Kami-dokan-bori Moat between 1997 and 1999, was not found during the present survey. Principal component analysis combining past and current survey results revealed distinct fish communities across different areas of the Imperial Palace Inner Garden. Among these, the fish community in the Dokan-bori Moat system showed relatively large yearly variations, suggesting a possible link to the gradual stagnation and partial land reclamation occurring in the system.

Keywords: freshwater fishes, metabarcoding analyses, species list, specimen collection, visual observation.

Introduction

The Imperial Palace (35˚41'N, 139˚45'E) in Chiyoda Ward is the largest green space remaining in the Tokyo metropolitan center. Edo Castle (predecessor of the Imperial Palace) was constructed by Lord Dokan Ota in the mid 15th Century, on the eastern edge of the Musashino Plateau. By the early Edo period, it was surrounded by an inlet "Chidori-ga-fuchi River" that extended eastward from the western valley, passing through the castle area to Hibiya Estuary, the downstream waterway being converted to the inner moats of the castle by Ieyasu Tokugawa, who organized the Edo Shogunate Government in 1603 (Ganzawa, 1985). The inner moats were largely completed around 1608 (Tokyo Metropolitan Library, 2011–), and the outer moats completed around 1636 (Kanai, 1981).

The National Museum of Nature and Science has conducted fauna and flora surveys of the Imperial Palace Inner Garden (surrounded by the Imperial Palace Outer Garden) every ca. ten years, Phase I being from FY1996–2000, Phase II from FY2009–2013 and Phase III from FY2021–2025. Since Phase II emphasis on temporal changes in the biota has been added (Kuramochi *et al*., 2014). Fish fauna survey was conducted in Phase I and Phase III with a quarter-century interval.

The Edo Castle moats feature a triple ring layout: inner moats in the Imperial Palace Inner Garden ("inside" inner moats), inner moats in the Imperial Palace Outer Garden ("outside" inner moats), and outer moats reaching Yotsuya (west) and Kanda River (east). The first-mentioned were the subject of this survey.

Within the Imperial Palace Inner Garden, eight large "inside" inner moats and multiple small ponds exist. The first fish fauna survey was conducted by the Garden Division of the Imperial Household Agency in 1986–1987 in the former moats and two ponds, recording 14 species belonging to seven families (Shinohara *et al*., 2026). Although voucher specimens collected at that time had long been considered as "missing," they were rediscovered in 2024 on a shelf of unsorted items in the Biological Laboratory of the Imperial Palace (BLIP). They included 23 specimens in total, in eight species of seven genera, representing five families (Shinohara *et al*., 2026). The Phase I fish fauna survey (1997–1999) was focused on three moats: Kami-dokan-bori, Naka-dokan-bori and Shimo-dokan-bori moats. The three Dokan-bori moats had originally existed as a single moat, possibly from the time of Lord Dokan Ota. In particular, the Kami-dokan-bori Moat, which was almost isolated from the downstream moats during the early Meiji Era was especially expected to have been maintained in its original condition (Matsuura *et al*., 2000). Matsuura *et al*. (2000) reported 11 species/subspecies belonging to six families from the three moats.

The Phase III survey was conducted with the addition of the five remaining moats and small ponds to the Dokan-bori moats, in order to understand the present faunal status and changes over the 40 year period since the first survey in 1986–1987. Importance was placed on covering almost the entire area of each moat/pond, as well as recording the locations of individual fishes, which had not been realized previously. A new recording method, metabarcoding analyses on environmental DNA in the waterbodies, was adopted in anticipation of future effort-saving faunal monitoring.

Materials and Methods

Field location

Field surveys were carried out in spring and autumn 2024, and summer 2025. Targeted waterbodies included eight "inside" inner moats and four ponds with a stream (Figs.1–3, Appendix I). Sampling areas included the East Garden and Fukiage Garden. Because the former area was open to the public in daytime each Sunday, Tuesday to Thursday and Saturday, surveys were conducted on Monday and Friday so as to not inconvenience visitors. Hirakawa-bori, Tenjin-bori and Hakucho-bori moats and Ninomaru-ike Pond are located in/around the East Garden. Fukiage Garden, located in the westernmost part of the Imperial Palace Inner Garden, was entirely private and restricted to the Imperial Family, requiring special permissions for the survey. The latter included four waterbodies: Fukiage Ohike Pond, Kaintei Pond, Kanbakutei Nagare Stream and Fukiage Benten-ike Pond, all of which (except Kaintei Pond) had been surveyed in 1986. Fukiage Ohike Pond, which was artificially constructed during the Edo Era (Biological Laboratory of Imperial Household, 1989), is located in front of the Emperor's residence (Fukiage Palace). The aforementioned three Dokan-bori moats, and Hasuike-bori and Inui-bori moats are located in the lower valley area, developed from the original basin of the Chidori-ga-fuchi River (Suzuki, 1975), extending between the East Garden and Fukiage Garden,

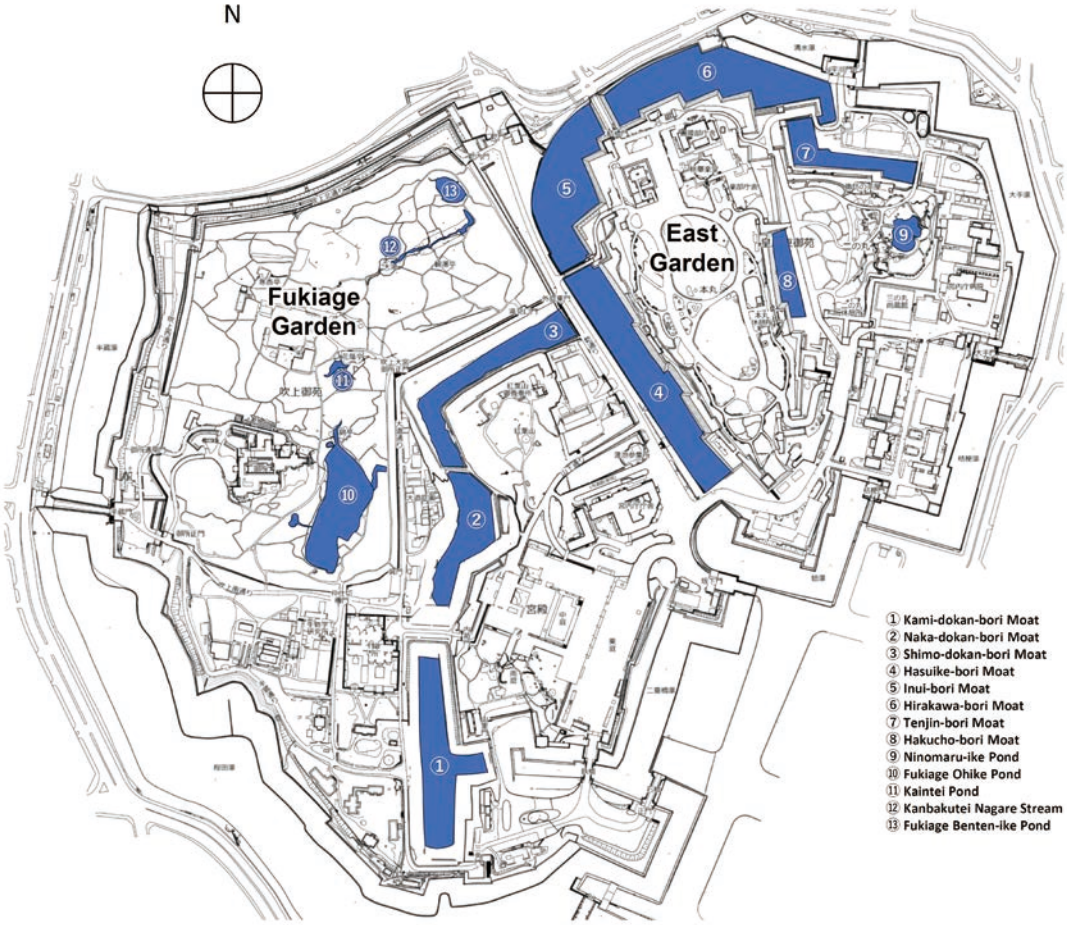

Fig. 1. Location of moats and ponds in Imperial Palace Inner Garden.

Fish Survey

Fishes were collected by hand net, casting net, set net, trammel net, hook & line, and in fish traps. A rope ladder was used to access the Hasuike-bori and Inui-bori moats, the water levels being considerably lower than the elevation of the nearest road. The trammel nets and set nets were deployed by survey team members so as to intersect fish swimming paths. Hand nets were used either to chase mid-moat fishes through the trammel or set nets, or to catch individual nearby fishes. In the largest moat (Hirakawa-bori Moat), an inflatable raft was used to deploy fish traps at the more-distant points, and to catch mid-moat fishes by hook & line. The fishes collected were photographed in the field using a digital camera, identified (mainly based on Nakabo, 2013), with individual numbers per species counted and recorded as accurately as possible, and their identifications subsequently confirmed in the laboratory.

During the 2024 spring survey, most of the fishes collected were retained as voucher specimens, thereby laying a foundation for future monitoring. Each was fixed in 10% formalin and later preserved in 70% ethanol for morphological observation or placed in 99% ethanol and stored frozen for DNA analyses (as tissue sample or whole body). During the 2024 autumn and 2025 summer surveys, which were undertaken to fill in gaps between collection-based and eDNA-based faunal results, the fishes collected were photographed, identified, counted and subsequently released [except for "new" records, including newly-collected species for the waterbody in 2024–2025, or a larger/smaller individual (beyond the size range recorded for the species in the 2024 spring survey)].

Fish locations were recorded by helmet-mounted GPS units and/or handwritten on maps, modelled on the preliminary fish survey in the Dokan-bori moats by NEWJEC Inc., commissioned by the Garden Division, Imperial Household Agency (NEWJEC, 2023). Identifications of larger fishes difficult to be captured were confirmed by field photographs, and individual numbers estimated from direct censuses taken by multiple observers standing at different points (using binoculars when necessary).

Complementing the voucher collection, fish specimen donations were received from the Parasitological Team (Team Leader: Toshiaki Kuramochi) surveying the Imperial Palace Inner Garden, including *Pseudorasbora* specimens collected in Fukiage Ohike Pond in 2021, and *Silurus asotus* and *Channa argus* collected in Tenjin-bori Moat in May to July 2024. Examples of *Pseudorasbora parva* collected in Hirakawa-bori Moat, and *Carassius* sp. and *Silurus asotus* collected in Tenjin-bori Moat in Oct. 2024 were received from the Kokyogaien National Garden Office (Ministry of the Environment).

All specimens were deposited in the Fish Collection, Department of Zoology, National Museum of Nature and Science, Tsukuba Campus, Ibaraki (NSMT-P). Abbreviations of standard length (SL) and total length (TL) are used throughout. Lateral line scales were examined using the Cyanin Blue staining method (Saruwatari *et al*., 1997). Classification follows Nakabo (2013) and Hosoya (2025).

Environmental DNA Survey

Sampling of eDNA. Metabarcoding was conducted on eDNA samples collected from 22 May 2024 to 11 July 2025 from moats and ponds in the Imperial Palace Inner Garden. Surface water samples (ca. 1.0 liter) were collected in sterilized plastic bottles, the sampling locations (1–5 per waterbody per day) being selected so as to represent the entire waterbody area, considering normal daily water flow. To prevent eDNA degradation during transport, 1 mL of 10% benzalkonium chloride solution (final concentration 0.01%) was added to the water samples immediately after collection, and thoroughly mixed by inversion. A control sample (field blank) collected on each sampling date served to demonstrate the absence of contaminants introduced during sampling. The sampling procedure generally followed the eDNA sampling and experiment manual (The eDNA Society, 2022, 2024). Instead of cleaning and reusing sampling gear, new (unused) plastic bottles and polypropylene ropes were used at each location. All samples were shipped under refrigerated conditions to IDEA Consultants, Inc. (Japan), where filtration, eDNA extraction, amplicon library construction, and sequencing were performed, following the procedures (including **Amplicon library and MiSeq sequencing** and **Bioinformatics**) (M. Nakamura, personal

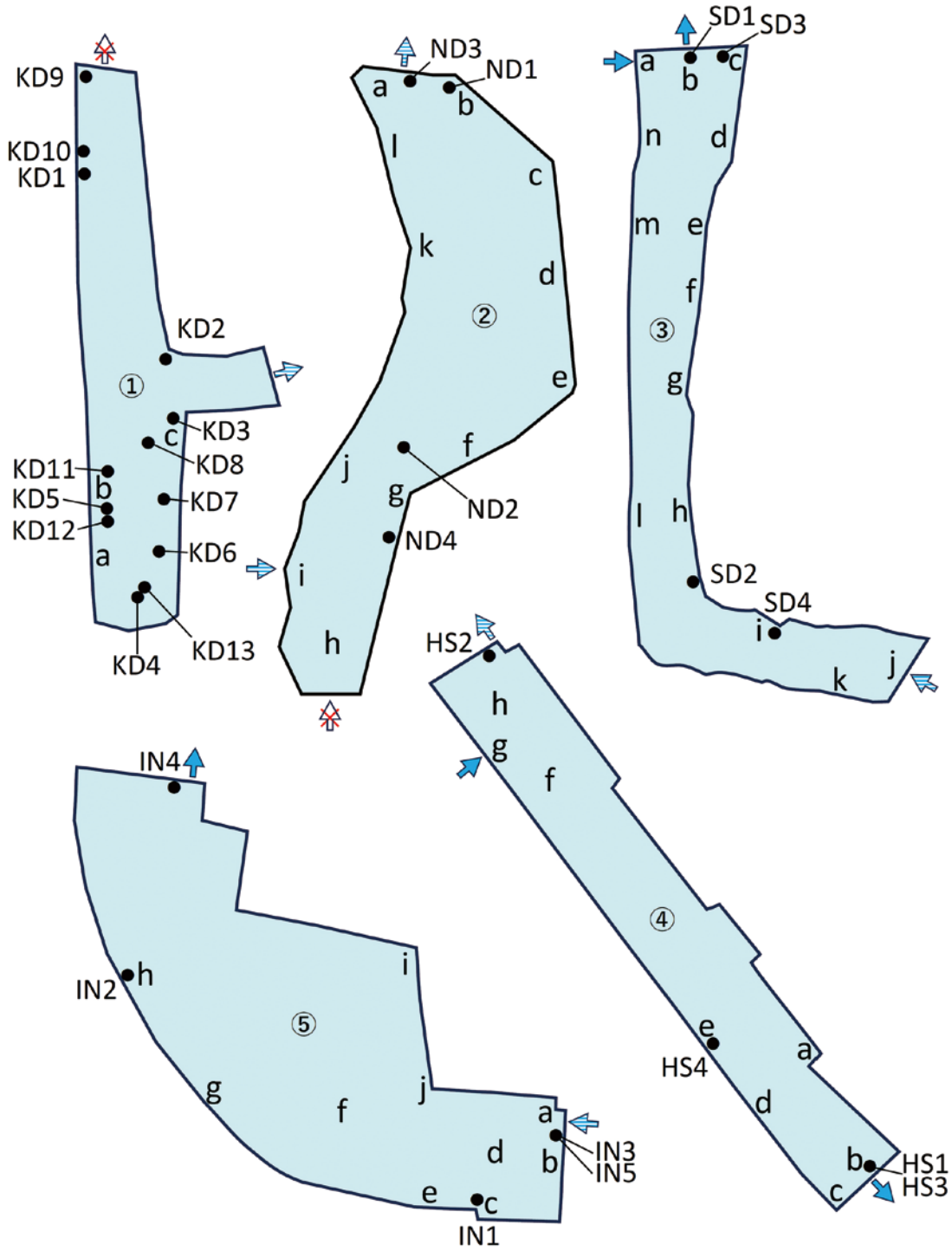

Fig. 2. Fish occurrence locations recorded in five moats. ①, Kami-dokan-bori Moat; ②, Naka-dokan-bori Moat; ③, Shimo-dokan-bori Moat; ④, Hasuike-bori Moat; ⑤, Inui-bori Moat. Numbered circles and lowercase letters correspond to Fig. 1 and Table 1, respectively. Alphanumeric codes (AA#) indicate eDNA water sampling stations. Arrows indicate inlet/outlet water passage and direction; color patterns indicate water flow status (see Fig. 10).

communication).

Water samples were filtered through a 47-mm diameter GF/F filter (nominal pore size, 0.7 μm; Cytiva, Whatman) within 24 hours of collection. To minimize cross-contamination, filter holders and other filtering equipment were bleached, rinsed with ultrapure water, and dried before and after filtration.

Total eDNA was extracted from each filter using a DNeasy® Blood & Tissue kits (Qiagen), taking care to avoid contamination [wearing lab gloves, cleaning with DNA AWAY™ Surface Decontaminant, and following other procedures described by the eDNA Society (2022, 2024)]. All samples were eluted in 200 μL of elution buffer, including the kits. For samples that did not amplify in the first PCR, potential PCR Inhibitors were removed using GeneReleaser® (Bioventures). All eDNA extracts were stored at −20° C until further analysis.

Amplicon library and MiSeq sequencing. Amplicon libraries of partial 12S rRNA gene were obtained by PCR amplification using MiFish universal primers (MiFish-U-F/R, MiFish-Ev2-F/R) (Miya *et al*., 2015). To ensure detection of species of Petromyzontidae, Siluriformes, Osmeridae, Plecoglossidae and Synbranchidae, some additional primers (MiFish-L-F/R, MiFish-05-F, MiFish-06-F, MiFish-09-F/04-R) were applied, which modified a few mismatches (see Biodiversity Center of Japan, 2021).

IDEA prepared an amplicon library following the protocol described by the eDNA Society (2024). The first PCR was performed using the two universal primer pairs, with a total reaction volume of 12 μL (6.0 μL 2 x AccuStart II PCR ToughMix (Quantabio), 1.2 μL MiFish primers mix at 10 uM, 2 μL sample DNA, and H_2O). The thermal cycle profile was 95 °C for 3 min; 35 cycles of 94°C for 30 s, 65 °C for 15 s, and 72 °C for 30 s. The first PCR products were diluted 10 times using Milli-Q water, and used as a template for the following PCR. The second PCR was performed to add MiSeq adaptor sequences and 8-bp unique dual indexes to both amplicon ends. The total reaction volume of the second PCR was also 12μL [6.0μL 2 x KAPA HiFi HotStart ReadyMix (KAPA Biosystems), each 2 μL of forward and reverse primers at 3.5 uM, 2 μL template DNA, and H_2O]. The thermal cycle profile for the second PCR was 95 °C for 3 min; 10 cycles at 98 °C for 20 s and 72 °C for 15 s; and 72 °C for 5 min. PCR amplifications were performed for 8 replicates for each eDNA sample.

After the second PCR, amplicons were repurified using AMPure XP Beads (Beckman Coulter), the concentrations of each PCR product being measured using the QuantStudio3 real-time PCR system (Thermo Fisher Scientific). All indexed PCR products were pooled in equal volume, and the pooled libraries purified by SPRI-select DNA Size Selection Reagent (Beckman Coulter). Finally, IDEA performed paired-end sequencing (2 x 150 bp), following the manufacturer's protocol using the MiSeq Reagent Kit v2 (Illumina).

Bioinformatics. Initially, FASTQ files were generated using bcl2fastq software (Illumina), while evaluating the Q-score values of the index sequences to ensure accurate demultiplexing. The Qiime2 pipeline was used to process all of the FASTQ files. Cutadapt was deployed to trim the primer sequences at the first stage. DADA2 was used for filtering, merging, and denoising all sequences. Chimeric sequences were also removed using the consensus method in DADA2, resulting in Amplicon Sequence Variance (ASVs). The ASV sequences were then assigned to taxonomy using BLAST+ with ≥98.5% similarity value at the species level.

Principal Component Analysis

Based on the fish faunal data (presence/absence) for the respective waterbodies in 1986–1987 (mainly documented in the 1986 report, see Shinohara *et al*., 2026), in 1997–1999 (documented in Matsuura *et al*., 2000), in 2024–2025 (documented herein), and eDNA faunal data (presence/absence, documented herein), a Principal Component Analysis (PCA) was performed from two perspectives: similarity/dissimilarity of occurrence tendency among fish species; and similarity/dissimilarity of fish fauna among 13 waterbodies and three survey periods (1986, 2000 and 2026). The target species were those collected or observed at least once during the overall period (1986–2026).

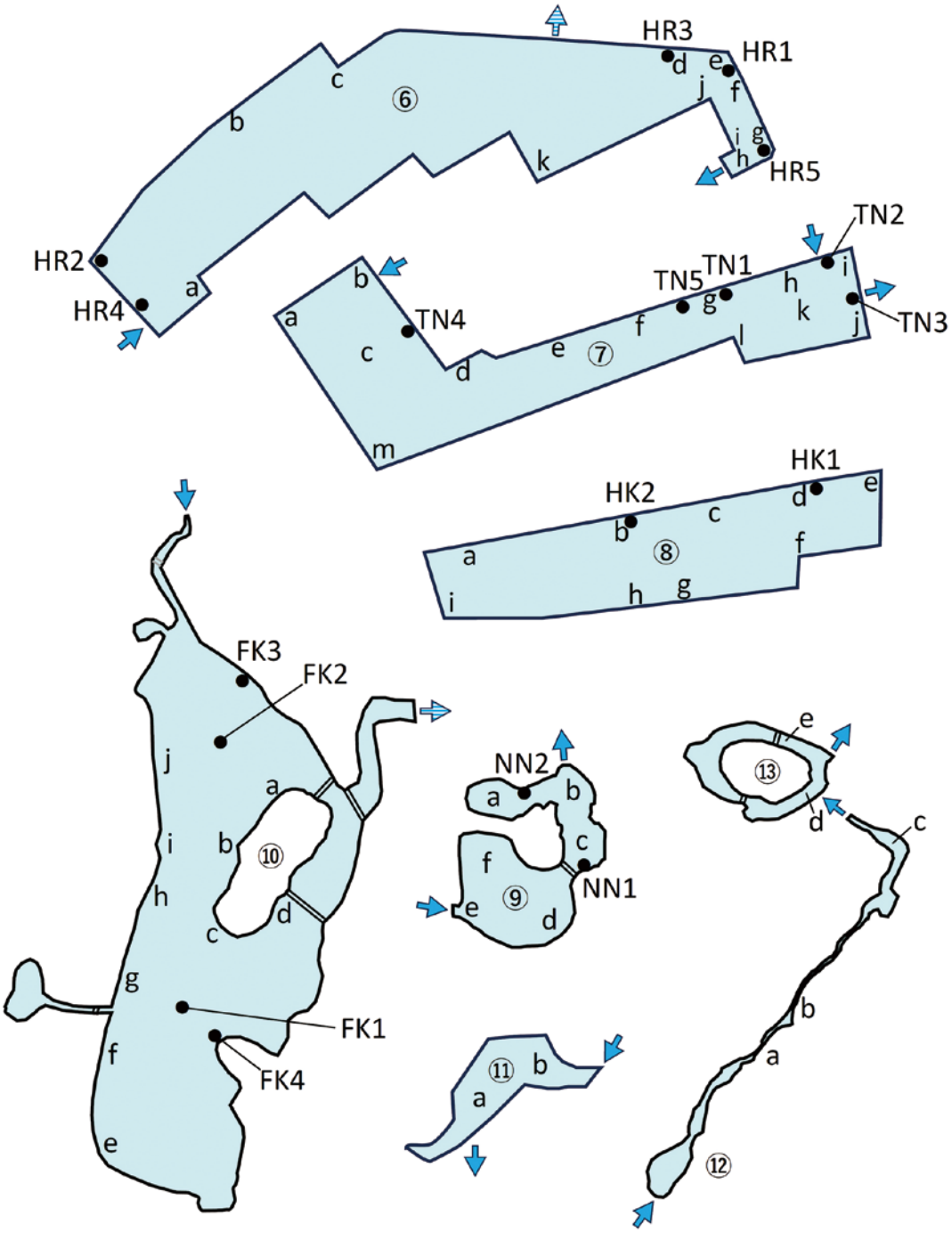

Fig. 3. Fish occurrence locations recorded in three moats, four ponds and a stream. ⑥, Hirakawa-bori Moat; ⑦, Tenjin-bori Moat; ⑧, Hakucho-bori Moat; ⑨, Ninomaru-ike Pond; ⑩, Fukiage Ohike Pond; ⑪, Kaintei Pond; ⑫, Kanbakutei Nagare Stream; ⑬, Fukiage Benten-ike Pond Numbered circles, lowercase letters, alphanumeric codes and arrows as in Fig. 2.

Species List

The field survey is summarized in Table 1 (see also Figs. 2–3); eDNA results are shown in Tables 2–4.

Fifteen collected and otherwise-observed species are listed with remarks.

Cypriniformes
Cyprinidae

Carassius sp.
[Japanese name: Ginbuna]
(Fig. 4A)

Collected from seven waterbodies; detected in nine (Table 5).

Specimens. *n* = 26, 4.0–31.9 cm SL.

Shimo-dokan-bori Moat (*n* = 3): NSMT-P 149358 (1), 11.1 cm SL, hand net, 10 June 2024; NSMT-P 150658 (1), 15.6 cm SL, hand net, 6 Nov. 2024; NSMT-P 150659 (1), 15.0 cm SL, hand net, 6 Nov. 2024.

Hasuike-bori Moat (*n* = 6): NSMT-P 149438 (1), 4.0 cm SL, set net, 26 June 2024; NSMT-P 150647 (1), 6.9 cm SL, hand net, 6 Nov. 2024; NSMT-P 150648 (4), 4.8–6.1 cm SL, hand net, 6 Nov. 2024.

Inui-bori Moat (*n* = 1): NSMT-P 150582 (1), 23.5 cm SL, trammel net, 28 Oct. 2024.

Hirakawa-bori Moat (*n* = 6): NSMT-P 150618 (1), 21.1 cm SL, trammel net, 1 Nov. 2024; NSMT-P 150619 (1), 17.6 cm SL, trammel net, 1 Nov. 2024; NSMT-P 150620 (1), 17.9 cm SL, trammel net, 1 Nov. 2024; NSMT-P 150627 (1), 11.7 cm SL, trammel net, 1 Nov. 2024; NSMT-P 150628 (1), 11.2 cm SL, casting net, 1 Nov. 2024; NSMT-P 150631 (1), 12.1 cm SL, trammel net, 1 Nov. 2024.

Tenjin-bori Moat (*n* = 2): NSMT-P 150580 (1), 8.3 cm SL, fish trap, 10 Oct. 2024; NSMT-P 150614 (1), 6.9 cm SL, hand net, 1 Nov. 2024.

Hakucho-bori Moat (*n* = 6): NSMT-P 149307 (1), 9.9 cm SL, casting net, 27 May 2024; NSMT-P 150568 (1), 12.4 cm SL, trammel net, 28 Oct. 2024; NSMT-P 150570 (1), 12.3 cm SL, hook & line, 28 Oct. 2024; NSMT-P 150575 (1), 14 cm SL, hook & line, 28 Oct. 2024; NSMT-P 150576 (1), 17.9 cm SL, hook & line, 28 Oct. 2024; NSMT-P 150577 (1), 17.7 cm SL, hook & line, 28 Oct. 2024.

Fukiage Ohike Pond (*n* = 2): NSMT-P 149436 (1), 31.8 cm SL, trammel net, 26 June 2024; NSMT-P 149437 (1), 31.9 cm SL, trammel net, 26 June 2024.

Remarks. This species was abundantly distributed in Kami-dokan-bori and Naka-dokan-bori moats (Matsuura *et al*., 2000). Maeda *et al*. (2003) reported its gynogenesis with 15 clones, including seven major clones from three Dokan-bori moats, based on 201 specimens (see also Shinohara *et al*., 2026).

The present survey did not capture or visually observe any individuals in Kami-dokan-bori Moat, in which the waterbody had decreased significantly since the 1998–1999 survey. However, the metabarcoding analysis from Kami-dokan-bori Moat was positive for this species.

Eighteen small unidentified specimens (*Carassius* sp. indet., 1.2–3.3 cm SL) (listed in Appendix II) were likely either *Carassius* sp. "Ginbuna" or *C. cuvieri*.

Carassius cuvieri Temminck and Schlegel, 1846
[Japanese name: Gengorobuna]
(Fig. 4B)

Collected from three waterbodies; detected in six (Table 5).

Specimens. *n* = 4, 9.5–24.7 cm SL.

Shimo-dokan-bori Moat (*n* = 1): NSMT-P 150662 (1), 12.0 cm SL, trammel net, 6 Nov. 2024.

Tenjin-bori Moat (*n* = 1): NSMT-P 150609 (1), 10.4 cm SL, trammel net, 1 Nov. 2024.

Hakucho-bori Moat (*n* = 2): NSMT-P 149308 (1), 9.5 cm SL, casting net, 27 May 2024; NSMT-P 150569 (1), 24.7 cm SL, trammel net, 28 Oct. 2024.

Remarks. This species has been introduced nationwide from Lake Biwa and the Yodogawa River System (Hosoya, 2013).

According to Shinohara *et al*. (2026), 182 *C. cuvieri* individuals were collected from eight waterbodies within the Imperial Palace Inner Garden in 1986. However, only four individuals collected from the above three locations during the present survey suggests an environmental change. The metabarcoding analyses matched the collection data for the three moats (Table 5).

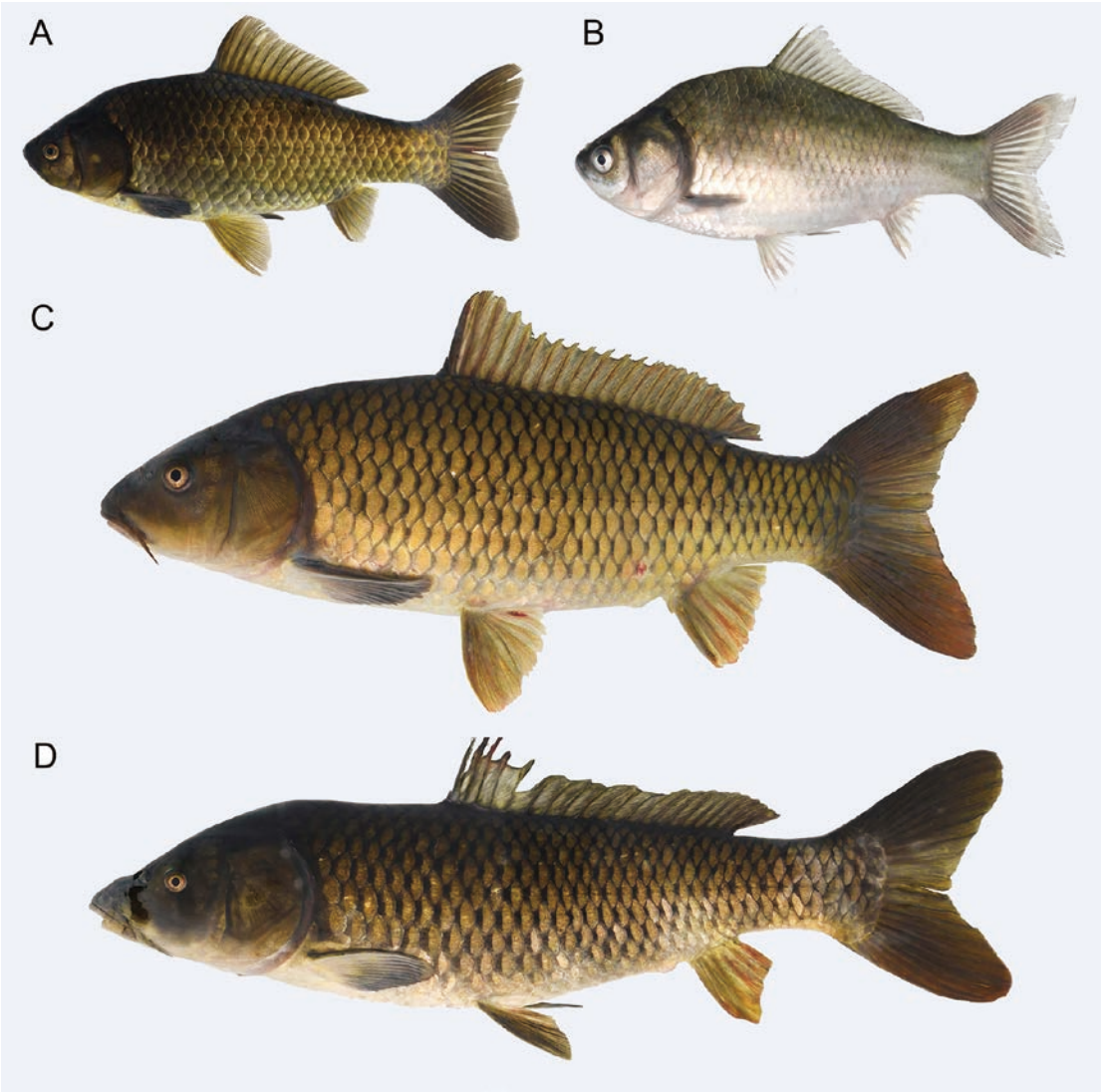

Fig. 4. Fresh specimens collected from Imperial Palace Inner Garden, Tokyo, Japan in 2024–2025. A, *Carassius* sp. "Ginbuna," NSMT-P 149436, 317.5 mm SL, Fukiage Ohike Pond; B, *Carassius cuvieri*, NSMT-P 150569, 247.0 mm SL, Hakucho-bori Moat; C, *Cyprinus carpio*, resembling reared type, NSMT-P 150637, 371.3 mm SL, Shimo-dokan-bori Moat; D, *C. carpio*, resembling wild type, NSMT-P 150608, 596.5 mm SL, Hirakawa-bori Moat.

Cyprinus carpio Linnaeus, 1758
[Japanese name: Koi]
(Figs. 4CD, 5A)

Collected from six waterbodies; observed in one; detected in eight (Table 5).

Specimens. *n* = 69, 1.7–44.4 cm SL

Naka-dokan-bori Moat (*n* = 2): NSMT-P 149344 (1), 40.9 cm SL, hand net, 10 June 2024; NSMT-P 149367 (1), 3.7 cm SL, fish trap, 10 June 2024.

Shimo-dokan-bori Moat (*n* = 2): NSMT-P 150583 (1), 16.1 cm SL, hook & line, 30 Oct. 2024; NSMT-P 150637 (1), 37.1 cm SL, trammel net, 6 Nov. 2024.

Hasuike-bori Moat (*n* = 1): NSMT-P 150650 (1),

Table 1. Survey methods and occurrence locations of fish species collected or observed in Imperial Palace Inner Garden moats and ponds.

Species names	① Kami-dokan-bori Moat (KD)	② Naka-dokan-bori Moat (ND)	③ Shimo-dokan-bori Moat (SD)	④ Hasuike-bori Moat (HS)	⑤ Inui-bori Moat (IN)	⑥ Hirakawa-bori Moat (HR)	⑦ Tenjin-bori Moat (TN)
Carassius sp. "Ginbuna"	—	—	**HN:** j-3(3).	**HN:** g-9(5). **SN:** g-1(1).	**TN:** e~j-1(1).	**CN:** f-1(1). **TN:** d~j-3(3), g~i-2(2).	**FT:** g-1(1). **HN:** a~b-1(1).
Carassius cuvieri	—	—	**TN:** m~e-1(1).	—	—	—	**TN:** g~l-1(1).
Carassius sp. indet.	—	—	**FT:** j-1(0). **SN:** b-1(1).	**SN:** g-1(1).	**FT:** c-1(1). **HN:** d-6(6). **SN:** a-5(5).	**CN:** g-1(1). **HN:** h-1(1). **SN:** i-1(1).	—
Cyprinus carpio	—	**FT:** b-1(1). **HN:** g-2(1), j-1(0).	**HL:** j-1(1). **TN:** n~d-4(1).	**HN:** g-1(1).	**HN:** b-1(1), d-3(3). **SN:** a-23(23).	**HN:** h-21(21). **SN:** i-5(5). **TN:** d~j-1(1), g~i-2(2).	**HL:** a-2(0). **HN:** k-8(8).
Ctenopharyngodon idellus	—	—	—	—	**OB:** d~f~h-5≦(0).	—	**OB:** a~c~m-1≦(0).
Hypophthalmichthys molitrix	—	—	—	—	—	—	**OB:** c~d~e~f-7≦(0).
Pseudorasbora parva	—	**FT:** a-45(45), b-17(17), d-3(3), e-3(3), i-5(5), j-1(0). **HN:** a-1(1), b-1(1), c-1(0), f-12(12), h-15(15), l-3(3). **SN:** k-43(43).	**FT:** a-6(0), h-55(0), j-49(7). **HN:** f-4(1), j-13(13). **SN:** l-13(13).	**FT:** b-32(32), e-6(0), f-13(13), h-14(14). **HN:** b-1(1), c-5(5), g-23(23). **SN:** g-82(59).	**FT:** a-13(0), b-17(0), c-13(1), g-2(2), h-1(1). **HL:** c-1(1). **HN:** d-3(3). **SN:** a-74(74).	**FT:** a-1(1), b-1(1), c-1(0), k-2(2). **HL:** d-1(1). **SN:** i-3(3).	**CN:** b-1(1). **FT:** g-2(1). **HL:** a-1(0), b-1(1). **HN:** a~b-10(0), i-5(5).
Pseudorasbora pumila	—	—	—	—	—	—	—
Misgurnus anguillicaudatus	**HN:** a-14(9), c-5(0)	—	**FT:** j-1(1).	**HN:** c-4(4)	—	—	—
Silurus asotus	—	—	—	—	—	**HN:** h-1(1).	**FT:** g-1(1). **HL:** i-2(2).
Gambusia affinis	—	**FT:** a-2(2). **HN:** b-2(2), c-2(2), f-1(1), h-30(30), l-1(1). **SN:** k-70(70).	**HN:** c-2(2), j-2(2), l-1(1)	**FT:** b-2(2), e-4(4), f-12(12), h-5(5). **HN:** a-1(1), c-13(13), g-11(11), **SN:** g-94(94).	**FT:** a-1(1). **HN:** d-1(1).	**HN:** e-4(4), h-2(2).	**HN:** m~a~b~d-41(41), k-1(1).
Gymnogobius castaneus	—	**FT:** a-47(47), b-8(8), **HN:** a-3(3), b-5(2), c-1(0), h-3(3), l-6(6). **SN:** k-27(27).	**CN:** b-10(10). **FT:** a-3(0), d-6(6), e-4(4), h-44(0), i-1(1), j-7(7). **HN:** a-30(0), d-2(2), f-20(4), g-5(5), j-2(2), l-10(10), n-6(6). **SN:** a-31(31), b-76(57), l-31(31).	**HN:** c-15(15), g-1(1). **SN:** g-7(7).	**FT:** c-4(4), g-1(1). **HN:** d-1(1). **SN:** a-26(26).	**CN:** g-2(2). **SN:** i-3(3).	**HN:** a~b-1(0), i-4(4), j-1(0), k-4(4). **SN:** l-1(1).
Gymnogobius urotaenia	—	**FT:** a-2(2), b-3(3). **HN:** a-1(1), l-1(1).	**CN:** b-1(1). **FT:** a-2(2), e-1(1), j-4(4). **HL:** j-1(1). **HN:** f-2(2), g-1(1), n-3(3). **SN:** a-1(1), b-8(7), l-18(18).	**HN:** g-2(2).	**SN:** a-2(2).	**CN:** g-2(2). **FT:** b-1(1). **HN:** h-6(6).	**FT:** b-2(0), e-3(3). **HN:** k-4(4). **SN:** h-1(0).
Rhinogobius kurodai	**HN:** b-2(2).	**FT:** a-1(1). **HN:** c-4(2).	**FT:** h-2(0). **HN:** a-2(2), f-3(0), g-1(1), k-1(1). **SN:** b-1(1), l-7(5).	**HN:** d-1(1), g-1(1).	**FT:** j-2(2). **HN:** d-2(2), i-1(1). **SN:** a-2(2).	**HN:** e-8(8), h-5(2). **SN:** i-3(3).	**FT:** i-1(1). **HN:** j-3(3).
Tridentiger brevispinis	—	—	—	—	—	**FT:** k-1(0). **HN:** h-1(1). **SN:** i-6(3).	**HL:** a-4(1).
Channa argus	—	—	—	**SN:** g-1(1).	**TN:** e~j-1(1).	**CN:** g-1(1). **TN:** g~i-2(1).	**HL:** i-2(2).

Table 1. Survey methods and occurrence locations of fish species collected or observed in Imperial Palace Inner Garden moats and ponds (continued).

Species names	⑧ Hakucho-bori Moat (HK)	⑨ Ninomaru-ike Pond (NN)	⑩ Fukiage Ohike Pond (FK)	⑪ Kaintei Pond (KI)	⑫ Kanbakutei Nagare Stream (KB)	⑬ Fukiage Benten-ike Pond (BN)
Carassius sp. "Ginbuna"	**CN:** c-1(1). **HL:** b-4(4). **TN:** b~h-1(1).	—	**TN:** b~i-2(2).	—	—	—
Carassius cuvieri	**CN:** c-1(1). **TN:** b~h-1(1).	—	—	—	—	—
Carassius sp. indet.	**HN:** g-1(1).	—	—	—	—	—
Cyprinus carpio	—	**OB:** a~b~c~d~e~f-17≤(0). (*C. carpio* x *C. carpio* var. *flavipinnis*)	—	—	—	—
Ctenopharyngodon idellus	—	—	—	—	—	—
Hypophthalmichthys molitrix	—	—	—	—	—	—
Pseudorasbora parva	**FT:** a-160(0), c-75(0), d-80(5). **HL:** b-1(1), d-1(1). **HN:** g-9(9).	**HN:** a~b~c~d~e~f-14(14).	**FT:** e-1(1). **SN:** i-1(1).	—	—	—
Pseudorasbora pumila	—	—	**FT:** e-35(35), h-3(3), i-20(20), j-2(2). **SN:** i-5(5).	—	—	—
Pseudorasbora sp. indet.			**FT:** a-1(1), e-7(7), h-1(1), i-8(8). **HN:** f-2(2). **SN:** i-36(36).			
Pseudorasbora probable hybrid *P. parva* x *P. pumila*			**FT:** f-1(1).			
Misgurnus anguillicaudatus	—	—	—	—	—	—
Silurus asotus	—	—	—	—	—	—
Gambusia affinis	—	**FT:** a-2(2), b-2(2), c-2(2), d-3(3). **HN:** a~b~c~d~e~f-6(6), e-8(8).	**FT:** c-1(1), e-9(9), f-2(2), i-5(5). **HN:** b~c~d-23(23), e-30(30), f-30(30), g-35(35). **SN:** i-3(3).	**HN:** a~b-323(323).	—	—
Gymnogobius castaneus	—	—	—	—	—	—
Gymnogobius urotaenia	**FT:** a-5(5). **HN:** d~e-1(1). **SN:** f-1(1).	—	—	—	—	—
Rhinogobius kurodai	**FT:** a-2(0), c-1(0). **HN:** d~e-10(7), i-1(1).	**FT:** a-1(1), b-2(2). **HN:** a~b~c~d~e~f-3(3), a-3(0).	—	—	**HN:** a-2(0), b-8(4), c-4(0).	**FT:** c-1(0). **HN:** d~e-8(6).
Tridentiger brevispinis	—	—	—	—	—	—
Channa argus	—	—	—	—	—	—

CN, casting net; **FT**, fish trap; **HL**, hook & line; **HN**, hand net, **OB**, observation; **SN**, set net; **TN**, trammel net.
Lowercase alphabet (a-n) indicates fish occurrence location in moats and ponds shown in Figs. 2–3.
"**TN:** b~c-3(2)" indicates three individuals collected by trammel net between points "b" and "c" in moat/pond, and two individuals fixed as voucher specimens among three.

Table 2. Results of metabarcoding analyses of waters sampled during May–June 2024.

			Number of detected reads																		
			22 May 2024						24 May 2024				27 May 2024				26 June 2024				
			Kami-dokan-bori Moat		Naka-dokan-bori Moat		Shimo-dokan-bori Moat		Inui-bori Moat		Hirakawa-bori Moat		Tenjin-bori Moat		Hakucho-bori Moat	Ninomaru-ike Pond	Hasuike-bori Moat		Fukiage Ohike Pond		Total reads
No.	Families	Species	St. KD1	St.KD2	St.ND1	St.ND2	St.SD1	St.SD2	St.IN1	St.IN2	St.HR1	St.HR2	St.TN1	St.TN2	St.HK1	St.NN1	St.HS1	St.HS2	St.FK1	St.FK2	
1	Anguillidae	*Anguilla japonica*	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	6
2	Cyprinidae	*Carassius* spp.	0	0	231	472	2,331	4,542	11,767	1,209	54,666	2,988	13,829	19,022	11,603	0	433	24,994	16,224	13,649	177,960
3	Cyprinidae	*Carassius cuvieri*	0	0	944	46	204	167	0	0	0	0	3,154	6,127	40,390	0	0	0	0	0	51,032
4	Cyprinidae	*Cyprinus carpio* (reared type)	2,127	0	8,850	34,056	126,300	5,920	4,673	933	5,293	149	42,461	36,045	0	79,372	48	1,153	0	0	347,380
5	Cyprinidae	*Cyprinus carpio* (wild type)	0	0	2,066	18,173	2,820	29	2,197	1,899	0	0	1,564	3,354	0	0	16	0	0	0	32,118
6	Cyprinidae	*Ctenopharyngodon idellus*	0	0	0	0	0	0	0	0	84	0	95	0	0	0	0	0	0	0	179
7	Cyprinidae	*Hypophthalmichthys* spp.	0	0	0	0	0	0	0	0	0	0	6,578	10,340	0	0	0	0	0	0	16,918
8	Cyprinidae	*Pseudorasbora* spp.	3,085	0	14,381	90,198	716	8,287	34,099	10,838	1,732	96	16,033	15,583	50,171	36,493	11,373	25,834	351	0	319,270
9	Cyprinidae	*Pseudorasbora pumila*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55,048	46,194	101,242
10	Cobitidae	*Misgurnus anguillicaudatus* (native)	0	0	0	0	0	10	0	0	0	0	0	0	0	0	90	224	0	0	324
11	Cobitidae	*Misgurnus anguillicaudatus* (non-native)	116,420	162,602	0	0	0	0	0	0	0	0	0	0	0	0	14	95	0	0	279,131
12	Cobitidae	*Misgurnus chipisaniensis*	0	0	9	340	4	610	0	0	0	0	0	0	0	0	0	10	0	0	973
13	Siluridae	*Silurus* spp.	0	0	0	0	0	19	213	584	1,111	16	3,232	7,996	0	0	0	0	0	0	13,171
14	Poeciliidae	*Gambusia affinis*	0	0	5,734	23,056	293	5,542	2,762	1,544	0	0	0	172	0	10,526	6,853	8,473	92,833	67,868	225,656
15	Gobiidae	*Gymnogobius* spp.	0	0	256,839	103,495	51,859	93,841	93,246	10,330	77,403	100,202	119,462	71,102	0	0	110,548	52,043	0	112	1,140,482
16	Gobiidae	*Gymnogobius urotaenia*	0	0	19	0	1,994	35,092	181	1,588	29,066	12,329	21,749	33,053	51,141	0	0	0	0	0	186,212
17	Gobiidae	*Rhinogobius* spp.	0	0	347	0	278	858	5,305	7,682	707	729	2,390	2,938	50,741	49,011	1,873	0	0	0	122,859
18	Gobiidae	*Tridentiger* spp.	3,342	0	0	0	0	0	0	0	788	224	53	420	0	0	0	0	0	0	4,827
19	Channidae	*Channa argus*	0	0	0	0	0	0	1,429	107,788	4,123	24,855	1,382	2,359	0	0	4,804	42,962	0	0	189,702
Total species in respective stations			4	3	12	10	12	14	12	12	12	12	15	15	7	6	12	11	6	6	
Total reads in respective stations			124,974	162,602	289,420	269,836	186,799	154,917	155,872	144,395	174,973	141,594	231,982	208,511	204,046	175,402	136,052	155,788	164,456	127,823	3,209,442

Table 3. Results of metabarcoding analyses of waters sampled during October–November 2024.

			Number of detected reads																		
			28 Oct. 2024				30 Oct. 2024						1 Nov. 2024				6 Nov. 2024				
			Inui-bori Moat		Hakucho-bori Moat	Ninomaru-ike Pond	Kami-dokan-bori Moat		Naka-dokan-bori Moat		Fukiage Ohike Pond		Hirakawa-bori Moat		Tenjin-bori Moat		Shimo-dokan-bori Moat		Hasuike-bori Moat		Total reads
No.	Family	Species	St.IN3	St.IN4	St.HK2	St.NN	St.KD3	St.KD4	St.ND3	St.ND4	St.FK3	St.FK4	St.HR3	St.HR4	St.TN3	St.TN4	St.SD3	St.SD4	St.HS3	St.HS4	
1	Anguillidae	*Anguilla japonica*	0	0	0	0	0	0	0	0	0	0	0	0	0	269	0	0	0	0	269
2	Cyprinidae	*Carassius* spp.	60,928	78,228	20,139	0	0	253	4,195	820	4,039	711	83,902	53,940	47,598	42,425	3,959	7,671	23,080	2,159	434,047
3	Cyprinidae	*Carassius cuvieri*	0	0	84,956	0	0	0	8,407	0	0	0	147	761	6,174	2,835	675	0	0	0	103,955
4	Cyprinidae	*Cyprinus carpio* (reared type)	10,918	13,756	0	44,697	0	0	36,768	68,751	0	0	26,425	4,148	21,884	38,621	11,057	9,322	5,745	22,200	314,292
5	Cyprinidae	*Cyprinus carpio* (wild type)	3,975	12,578	0	0	0	0	10,467	31,390	0	0	663	0	1,686	72	396	2,596	299	6,749	70,871
6	Cyprinidae	*Ctenopharyngodon idellus*	1,372	0	0	0	0	0	0	0	0	0	281	0	0	571	0	0	0	0	2,224
7	Cyprinidae	*Hypophthalmichthys* spp.	0	0	0	0	0	0	0	0	0	0	0	0	45	4,560	0	0	0	0	4,605
8	Cyprinidae	*Pseudorasbora* spp.	36,652	13,575	10,129	37,249	0	0	39,607	9,311	0	0	5,265	14,366	4,979	8,290	3,990	21,354	27,460	36,988	269,215
9	Cyprinidae	*Pseudorasbora pumila*	0	0	0	0	0	0	0	0	15,499	3,836	0	0	0	0	0	0	0	0	19,335
10	Cobitidae	*Misgurnus anguillicaudatus* (native lineage)	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	41	0	0	47
11	Cobitidae	*Misgurnus anguillicaudatus* (non-native lineage)	136	0	0	0	159,602	54,725	0	0	0	0	0	0	0	0	0	0	0	0	214,463
12	Cobitidae	*Misgurnus chipisaniensis*	0	0	0	0	0	0	1,330	590	0	0	0	0	0	0	0	116	0	0	2,036
13	Siluridae	*Silurus* spp.	1,998	2,424	0	0	0	0	0	0	0	0	3,426	6,866	14,626	7,802	15	0	0	0	37,157
14	Poeciliidae	*Gambusia affinis*	8,469	7,637	0	25,742	66	517	56,649	24,347	81,549	72,589	0	7,094	575	1,417	2,811	31,288	35,231	21,290	377,271
15	Gobiidae	*Gymnogobius* spp.	23,728	19,678	0	0	0	0	38,094	5,079	0	0	42,643	6,237	12,137	11,023	86,418	40,556	17,864	23,689	327,146
16	Gobiidae	*Gymnogobius urotaenia*	4,185	81	68,556	0	0	0	3,577	831	0	0	6,286	4,690	526	3,188	3,462	34,915	103	3,475	133,875
17	Gobiidae	*Rhinogobius* spp.	4,040	5,085	13,118	28,876	508	21,147	78	65	0	0	14,782	22,142	3,504	5,766	2,538	3,217	155	789	125,810
18	Gobiidae	*Tridentiger* spp.	0	0	0	0	0	0	0	0	0	0	6,162	5,853	0	427	0	0	0	0	12,442
19	Channidae	*Channa argus*	11,732	17,499	0	0	0	0	136	56	0	0	11,856	8,862	9,165	13,488	0	0	18,835	25,262	116,891
Total species in respective stations			12	10	5	4	3	4	11	10	3	3	12	11	13	15	10	10	9	9	
Total reads in respective stations			168,133	170,541	196,898	136,564	160,176	76,642	199,308	141,240	101,087	77,136	201,838	134,959	122,905	140,754	115,321	151,076	128,772	142,601	2,565,951

Table 4. Results of metabarcoding analyses of waters sampled in July 2025.

No.	Family	Species	Number of detected reads												Total reads
			7 July 2025				11 July 2025								
			Kami-dokan-bori Moat				Kami-dokan-bori Moat					Inui-bori Moat	Hirakawa-bori Moat	Tenjin-bori Moat	
			St.KD5	St.KD6	St.KD7	St.KD8	St.KD9	St.KD10	St.KD11	St.KD12	St.KD13	St.IN5	St.HR5	St.TN5	
1	Anguillidae	*Anguilla japonica*	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Cyprinidae	*Carassius* spp.	0	0	0	0	0	0	0	0	0	77,254	69,189	4,830	151,273
3	Cyprinidae	*Carassius cuvieri*	0	0	0	0	0	0	0	0	0	966	177	596	1,739
4	Cyprinidae	*Cyprinus carpio* (reared type)	0	0	0	0	0	0	0	0	0	14,595	5,527	24,638	44,760
5	Cyprinidae	*Cyprinus carpio* (wild type)	0	0	0	0	0	0	0	0	0	2,378	0	1,124	3,502
6	Cyprinidae	*Ctenopharyngodon idellus*	0	0	0	0	0	0	0	0	0	709	0	1,916	2,625
7	Cyprinidae	*Hypophthalmichthys* spp.	236	0	0	0	0	0	0	0	0	0	0	12,572	12,808
8	Cyprinidae	*Pseudorasbora* spp.	0	0	0	0	0	0	0	0	0	12,732	3,199	13,441	29,372
9	Cyprinidae	*Pseudorasbora pumila*	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Cobitidae	*Misgurnus anguillicaudatus* (native lineage)	0	0	0	0	0	0	0	0	0	0	0	0	0
11	Cobitidae	*Misgurnus anguillicaudatus* (non-native lineage)	175,129	182,623	266,669	19,500	0	0	6,352	160,554	199,128	0	0	0	1,009,955
12	Cobitidae	*Misgurnus chipisaniensis*	0	0	0	0	0	0	0	0	0	0	0	0	0
13	Siluridae	*Silurus* spp.	0	0	0	0	0	0	0	0	0	946	4,019	6,731	11,696
14	Poeciliidae	*Gambusia affinis*	557	0	234	0	0	62,939	302	26	24	3,106	0	185	67,373
15	Gobiidae	*Gymnogobius* spp.	0	0	0	0	0	0	0	0	0	21,926	50,254	47,207	119,387
16	Gobiidae	*Gymnogobius urotaenia*	0	0	0	0	0	0	0	0	0	15,068	5,703	33,593	54,364
17	Gobiidae	*Rhinogobius* spp.	575	0	0	12,803	0	0	0	21	0	20,316	15,874	23,001	72,590
18	Gobiidae	*Tridentiger* spp.	0	0	0	0	0	0	0	0	0	48	6,025	374	6,447
19	Channidae	*Channa argus*	0	0	0	0	0	0	0	0	0	15,625	15,913	2,797	34,335
Total species/lineage in respective stations			4	1	2	2	0	1	2	3	2	11	8	12	
Total reads in respective stations			176,497	182,623	266,903	32,303	0	62,939	6,654	160,601	199,152	107,449	106,514	167,579	1,622,226

Table 5. Summary of species collected/observed/detected in Imperial Palace Inner Garden moats and ponds during 1986–2025.

Families	Species	Kami-dokan-bori Moat	Naka-dokan-bori Moat	Shimo-dokan-bori Moat	Hasuike-bori Moat	Inui-bori Moat	Hirakawa-bori Moat	Tenjin-bori Moat	Hakucho-bori Moat	Ninomaru-ike Pond	Fukiage Ohike Pond	Kaintei Pond	Kambakutei Nagare Stream	Fukiage Benten-ike Pond
Anguillidae	*Anguilla japonica*						e	e						
Cyprinidae	*Carassius* sp. "Ginbuna"	abe	bce	abcde	de	de	ade	adef	de		ade			
Cyprinidae	*Carassius cuvieri*		abe	ade	a	ae	ae	ade	ade		a			
Cyprinidae	*Cyprinus carpio*	e	bcde	ade	ade	ade	de	ade		de	a			a
Cyprinidae	*Ctenopharyngodon idellus*				a	ade	ae	ade			a			
Cyprinidae	*Hypophthalmichthys molitrix*	e	b					ade	a					
Cyprinidae	*Pseudorasbora parva*	abe	abcde	abcde	ade	ade	ade	adef	ade	de	def			
Cyprinidae	*Pseudorasbora pumila*										def			
Cobitidae	*Misgurnus anguillicaudatus*	abcde		acde	de	e		e						
Cobitidae	*Misgurnus chipisaniensis*		e	e	e									
Siluridae	*Silurus asotus*			abe	b	e	ade	aef						
Adrianichthyidae	*Oryzias latipes*		ab	b							a			
Poeciliidae	*Gambusia affinis*	ce	acde	acde	ade	de	de	de		de	de	d		
Gobiidae	*Gymnogobius castaneus*		abcde	acde	ade	ade	ade	ade			e			
Gobiidae	*Gymnogobius urotaenia*		cde	acde	de	de	ade	de	de					
Gobiidae	*Rhinogobius kurodai*	abcde	acde	abcde	de	ade	ade	ade	ade	de			d	acd
Gobiidae	*Tridentiger brevispinis*	e				e	def	de						
Channidae	*Channa argus*		e		bde	ade	ade	ef						

a, Years 1986–1987 (Shinohara *et al*., 2026)
b, Years 1997–1999 (Matsuura *et al*., 2000)
c, Year 2022 (NEWJEC, 2023)
d, Years 2024–2025 (Present study: specimen collection, visual observation)
e, Years 2024–2025 (Present study: eDNA metabarcoding)
f, Years 2021–2024 (Donation from Parasitological Team and Ministry of the Environment)

44.4 cm SL, hand net, 6 Nov. 2024.

Inui-bori Moat (n = 27): NSMT-P 149072 (4), 1.9–2.8 cm SL, hand net, 24 May 2024; NSMT-P 149583 (23), 1.8–2.6 cm SL, set net, 24 May 2024.

Hirakawa-bori Moat (n = 29): NSMT-P 148983 (1), 3.5 cm SL, hand net, 24 May 2024; NSMT-P 148984 (1), 2.8 cm SL, hand net, 24 May 2024; NSMT-P 148985 (17), 2.6–3.6 cm SL, hand net, 24 May 2024; NSMT-P 149068 (2), 2.5–3.4 cm SL, hand net, 24 May 2024; NSMT-P 149077 (1), 51.1 cm SL, set net, 27 May 2024; NSMT-P 149078 (1), 39.3 cm SL, set net, 27 May 2024; NSMT-P 149328 (3), 2.3–3.4 cm SL, set net, 27 May 2024; NSMT-P 150608 (1), 59.7 cm SL, trammel net, 1 Nov. 2024; NSMT-P 150629 (1), 12.6 cm SL, trammel net, 1 Nov. 2024; NSMT-P 150630 (1), 12.2 cm SL, trammel net, 1 Nov. 2024.

Tenjin-bori Moat (n = 8): NSMT-P 149337 (8), 1.7–3.7 cm SL, hand net, 27 May 2024.

Remarks. According to Shinohara *et al*. (2026), 24 individuals were collected from the Shimo-dokan-bori, Hasuike-bori, Inui-bori and Tenjin-bori moats, and Fukiage Ohike and Fukiage Benten-ike ponds during 1986–1987. Most individuals encountered were shallow-bodied (resembling wild-form individuals). However, deep-bodied individuals were also collected from Fukiage Ohike and Fukiage Benten-ike ponds, strongly suggesting the presence of stocked and introduced populations. In addition, Matsuura *et al*. (2000) observed several Japanese ornamental carp (Nishikigoi) in Naka-dokan-bori Moat, but did not collect specimens.

The metabarcoding analyses matched all of the collection sites, with wild carp DNA being detected in the Naka-dokan-bori, Shimo-dokan-bori, Hasuike-bori, Inui-bori and Tenjin-bori moats. Although relationships between body depth and genetic types have been pointed out in Lake Biwa (Atsumi *et al*., 2017), such relationships are unclear in the Imperial Palace. The analysis also detected reared carp in the Kami-dokan-bori Moat, but no individuals were observed there.

In Ninomaru-ike Pond, longfin ornamental carp (Hirenaga-nishikigoi in Japanese) numbered at least 17 individuals, having originated from hybridization of Nishikigoi (Japanese ornamental carp) and Hirenagagoi (Indonesian longfin carp, *Cyprinus carpio* var. *flavipinnis*) at the Saitama Fisheries Research Institute, following a suggestion from Prince Akihito in 1977 (Fukuda, 1990; Fig. 5A).

Ctenopharyngodon idellus (Valenciennes, 1844)
[Japanese name: Sogyo]
(Fig. 5B)

Observed in two waterbodies; detected in three (Table 5).

Specimens. None.

Remarks. Specimens were not obtained, but large individuals were observed in Inui-bori and Tenjin-bori moats. Individual numbers were estimated as at least five in Inui-bori Moat (M. Hayashi, M. Kishida and G. Shinohara, personal observation on 4 June 2025) and one in Tenjin-bori Moat (M. Kishida, personal observation on 18 July 2025).

This species was introduced to Japan from mainland China after 1878 and released nationwide in order to remove lush aquatic plants (Hosoya, 2025). A total of 120 individuals (14–17 cm TL) were introduced to the inner moats of the Imperial Palace from the Saitama Fisheries Experimental Station (Saitama Fisheries Research Institute) on November 1957, having been reared from eggs from the introduced Tone River population (Suzuki *et al*., 1958).

Records exist of nine individuals in total collected from Inui-bori, Hirakawa-bori and Tenjin-bori moats in 1986, and two individuals transplanted from Inui-bori Moat to Hasuike-bori Moat, plus a visual record in Fukiage Ohike Pond in the same period (Shinohara *et al*., 2026). The individuals in Fukiage Ohike Pond were transplanted to Inui-bori and Hirakawa-bori moats in 1993, before extensive repair work (BLIP memo). The metabarcoding analyses detected eDNA from the Inui-bori, Hirakawa-bori and Tenjin-bori moats; however, no individuals were observed in Hirakawa-bori Moat.

Hypophthalmichthys molitrix (Valenciennes, 1844)
[Japanese name: Hakuren]
(Fig. 5C)

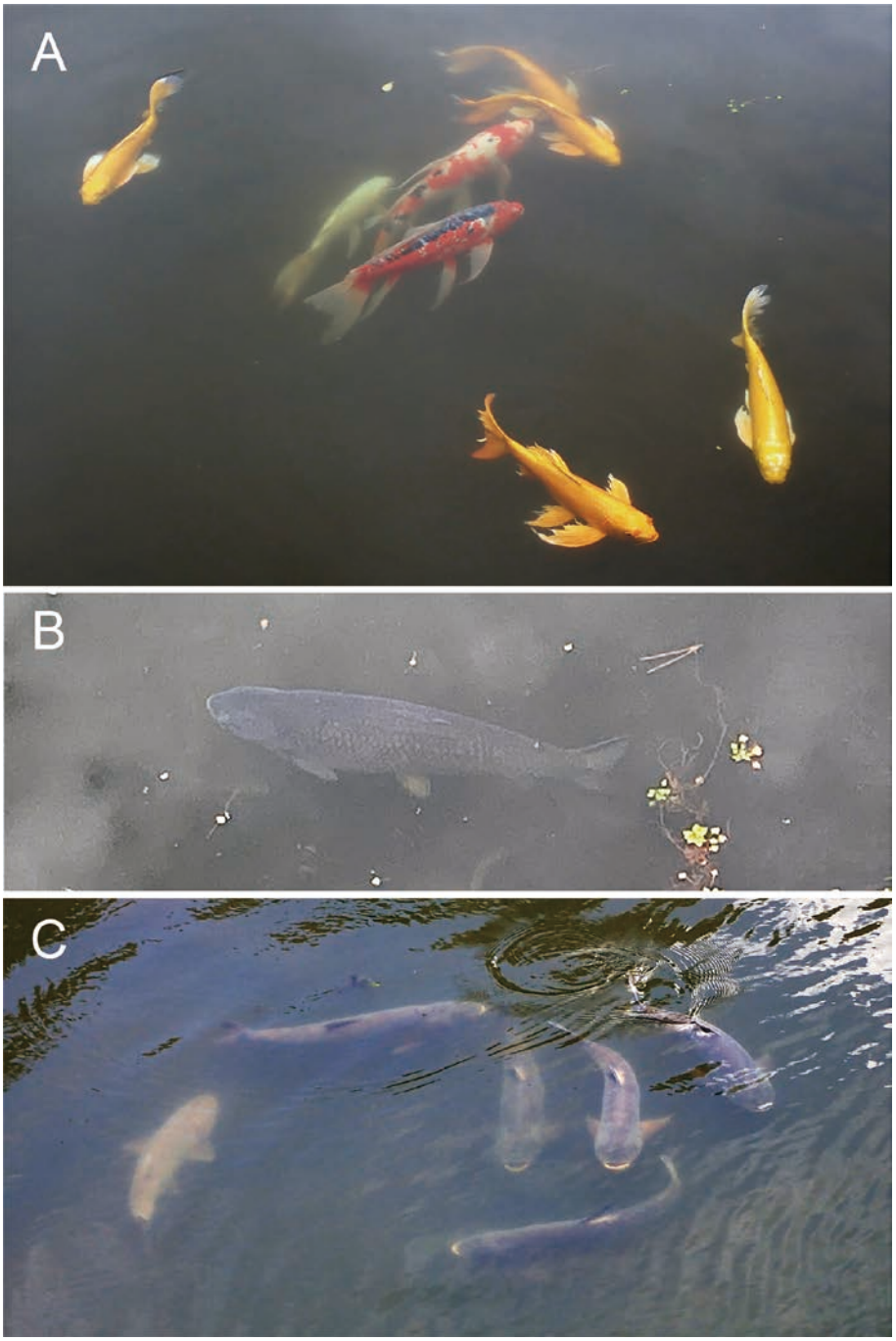

Fig. 5. Photographs of live fishes in Imperial Palace Inner Garden, Tokyo, Japan in 2024–2025. A, *Cyprinus carpio* x *Cyprinus carpio* var. *flavipinnis* (longfin ornamental carp—Hirenaga-nishikigoi) in Ninomaru-ike Pond (28 Oct. 2024); B, *Ctenopharyngodon idellus* in Inui-bori Moat (4 June 2025); C, *Hypophthalmichthys molitrix* in Tenjin-bori Moat (18 July 2025).

Observed in one waterbody; detected in two (Table 5).

Specimens. None.

Remarks. Six or seven large individuals were observed in Tenjin-bori Moat (M. Hayashi and M. Kishida, personal observation) on 4 June 2025, occasionally swimming together or swirling about (Fig. 5C).

This species, a phytoplankton feeder, was introduced to Japan from mainland China in late 1890, and released nationwide in order to improve water quality. A total of 120 individuals (13–17 cm TL) were introduced to the inner moats of the Imperial Palace (together with *C. idellus*) from the Saitama Fisheries Experimental Station (Saitama Fisheries Research Institute) on November 1957, having been reared from eggs collected from the introduced population in the Tone River (Suzuki *et al*., 1958). According to Shinohara *et al*. (2026), nine individuals were collected from the Tenjin-bori and Hakucho-bori moats in 1986. Subsequently Matsuura *et al*. (2000: Fig. 6) collected a single large specimen (76 cm SL) from Naka-dokan-bori Moat.

The metabarcoding analyses detected the species in Kami-dokan-bori and Tenjin-bori moats (Table 5).

Pseudorasbora parva (Temminck and Schlegel, 1846)
[Japanese name: Motsugo]
(Figs. 6AB, 7)

Collected from nine waterbodies; detected in ten (Table 5).

Specimens. *n* = 459, 1.5–8.5 cm SL.

Naka-dokan-bori Moat (*n* =148): NSMT-P 149056 (18), 1.7–2.3 cm SL, fish trap, 24 May 2024; NSMT-P 149361 (1), 1.7 cm SL, hand net, 10 June 2024; NSMT-P 149366 (3), 1.6–2.5 cm SL, hand net, 10 June 2024; NSMT-P 149370 (17), 2.2–3.8 cm SL, fish trap, 10 June 2024; NSMT-P 149371 (3), 2.1–4.0 cm SL, fish trap, 10 June 2024; NSMT-P 149372 (5), 2.1–3.9 cm SL, fish trap, 10 June 2024; NSMT-P 149373 (4) 2.4–3.1 cm SL, hand net, 10 June 2024; NSMT-P 149378 (12), 1.9–4.0 cm SL, hand net, 10 June 2024; NSMT-P 149379 (3), 2.2–3.7 cm SL, fish trap, 10 June 2024; NSMT-P 149380 (26), 1.5–5.2 cm SL, fish trap, 10 June 2024; NSMT-P 149585 (43), 1.9–3.8 cm SL, set net, 10 June 2024; NSMT-P 150584 (1), 5.5 cm SL, fish trap, 30 Oct. 2024; NSMT-P 150586 (12), 2.0–5.2 cm SL, hand net, 30 Oct. 2024.

Shimo-dokan-bori Moat (*n* = 34): NSMT-P 148934 (1), 4.8 cm SL, fish trap, 22 May 2024; NSMT-P 148935 (1), 4.3 cm SL, fish trap, 22 May 2024; NSMT-P 148936 (1), 5.2 cm SL, fish trap, 22 May 2024; NSMT-P 148947 (1), 5.0 cm SL, fish trap, 22 May 2024; NSMT-P 148950 (1), 4.3 cm SL, fish trap, 22 May 2024; NSMT-P 148951 (1), 3.9 cm SL, fish trap, 22 May 2024; NSMT-P 148968 (1), 2.1 cm SL, hand net, 22 May 2024; NSMT-P 148969 (1), 2.5 cm SL, fish trap, 22 May 2024; NSMT-P 149035 (1), 5.3 cm SL, set net, 24 May 2024; NSMT-P 149036 (5), 3.9–5.9 cm SL, set net, 24 May 2024; NSMT-P 149060 (7), 2.2–5.4 cm SL, set net, 24 May 2024; NSMT-P 149359 (1), 4.6 cm SL, hand net, 10 June 2024; NSMT-P 150655 (1), 5.9 cm SL, hand net, 6 Nov. 2024; NSMT-P 150660 (1), 7.7 cm SL, hand net, 6 Nov. 2024; NSMT-P 150661 (10), 4.1–7.9 cm SL, hand net, 6 Nov. 2024.

Hasuike-bori Moat (*n* = 147): NSMT-P 149357 (2), 2.0–2.7 cm SL, hand net, 10 June 2024; NSMT-P 149451 (1), 3.5 cm SL, fish trap, 26 June 2024; NSMT-P 149452 (2), 1.5–1.9 cm SL, fish trap, 26 June 2024; NSMT-P 149455 (14), 1.9–3.4 cm SL, fish trap, 26 June 2024; NSMT-P 149456 (13), 1.6–3.5 cm SL, fish trap, 26 June 2024; NSMT-P 149459 (24), 2.0–3.7 cm SL, set net, 26 June 2024; NSMT-P 149464 (23), 2.0–4.8 cm SL, hand net, 26 June 2024; NSMT-P 149468 (3), 2.0–2.5 cm SL, hand net, 26 June 2024; NSMT-P 149469 (29), 1.5–4.6 cm SL, fish trap, 26 June 2024; NSMT-P 150638 (35), 1.8–6.9 cm SL, set net, 6 Nov. 2024; NSMT-P 150643 (1), 2.3 cm SL, hand net, 6 Nov. 2024.

Inui-bori Moat (*n* = 82): NSMT-P 148974 (1), 6.2 cm SL, set net, 24 May 2024; NSMT-P 148975 (1), 5.6 cm SL, set net, 24 May 2024; NSMT-P 149073 (3), 1.9–2.2 cm SL, hand net, 24 May 2024; NSMT-P 149310 (1), 6.1 cm SL, fish trap, 27 May 2024; NSMT-P 149336 (3), 2.1–2.4 cm SL, fish trap, 27 May 2024; NSMT-P 149580 (72), 2.0–4.9 cm SL, set net, 24 May 2024; NSMT-P 150572 (1), 7.2 cm SL, hook & line, 28 Oct. 2024.

Hirakawa-bori Moat (*n* = 8): NSMT-P 150581 (2), 5.1–7.0 cm SL, fish trap, 10 Oct. 2024; NSMT-P 150622 (1), 6.8 cm SL, fish trap, 1 Nov. 2024;

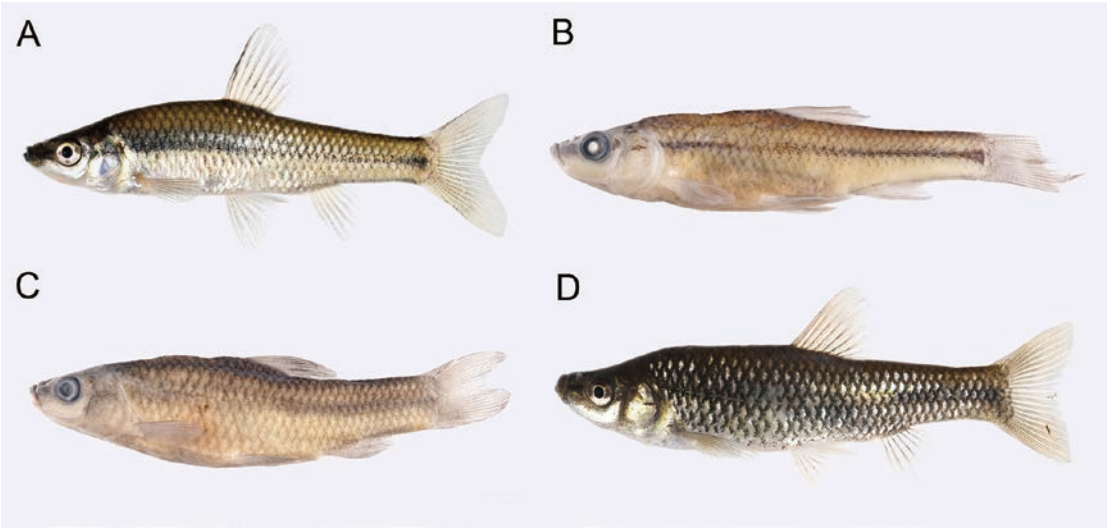

Fig. 6. Specimens of *Pseudorasbora* from Imperial Palace, Tokyo, Japan in 2021 and 2024–2025. A, *P. parva*, NSMT-P 148934, fresh condition, 47.6 mm SL, Shimo-dokan-bori Moat; B, *P. parva*, NSMT-P 153486, alcohol preserved, 40.4 mm SL, Fukiage Ohike Pond; C, *P. pumila*, NSMT-P 149445, alcohol preserved, 46.7 mm SL, Fukiage Ohike Pond; D, probable hybrid (*P. parva* x *P. pumila*), NSMT-P 150595, fresh condition, 46.2 mm SL, Fukiage Ohike Pond.

NSMT-P 150623 (1), 6.5 cm SL, hook & line, 1 Nov. 2024; NSMT-P 150626 (1), 7.5 cm SL, fish trap, 1 Nov. 2024; NSMT-P 150632 (3), 4.8–5.7 cm SL, set net, 1 Nov. 2024.

Tenjin-bori Moat (n = 8): NSMT-P 149311 (1), 6.4 cm SL, fish trap, 27 May 2024; NSMT-P 149313 (1), 2.5 cm SL, casting net, 27 May 2024; NSMT-P 150610 (1), 4.8 cm SL, hook & line, 1 Nov. 2024; NSMT-P 150615 (5), 3.9–5.5 cm SL, hand net, 1 Nov. 2024.

Hakucho-bori Moat (n = 16): NSMT-P 149075 (1), 6.5 cm SL, fish trap, 27 May 2024; NSMT-P 149076 (1), 5.9 cm SL, fish trap, 27 May 2024; NSMT-P 149309 (2), 7.1–8.2 cm SL, fish trap, 27 May 2024; NSMT-P 149315 (9), 2.2–2.7 cm SL, hand net, 27 May 2024; NSMT-P 150564 (1), 8.5 cm SL, fish trap, 28 Oct. 2024; NSMT-P 150571 (1), 6.8 cm SL, hook & line, 28 Oct. 2024; NSMT-P 150578 (1), 6.7 cm SL, hook & line, 28 Oct. 2024.

Ninomaru-ike Pond (n = 14): NSMT-P 149320 (14), 3.1–5.0 cm SL, fish trap, 27 May 2024.

Fukiage Ohike Pond (n = 2): NSMT-P 149441 (1), 3.5 cm SL, set net, 26 June 2024; NSMT-P 153486 (1), 4.0 cm SL, fish trap, 11 Aug. 2011.

Remarks. According to Shinohara *et al*. (2026), 3,560 individuals in total were collected during the 1986 survey, from the Kami-dokan-bori, Naka-dokan-bori, Shimo-dokan-bori, Hasuike-bori, Inui-bori, Hirakawa-bori, Tenjin-bori and Hakucho-bori moats.

This species is now widely distributed throughout Japan, except for parts of Hokkaido and Kyushu, having been originally distributed west of the Kanto Region, Honshu Island (Kawase and Hosoya, 2015). The Imperial Palace water systems could be included within the natural distribution of the species (Seigo Kawase, personal communication). No size distribution bias was observed among individuals in each waterbody, suggesting that they are reproducing within each moat (see Fig. 7B).

Pseudorasbora pumila Miyadi, 1930
[Japanese name: Shinai-motsugo]
(Figs. 6C, 7)

Collected from one waterbody; detected in same (Table 5).

Specimens. n = 65, 2.5–5.5 cm SL.

Fukiage Ohike Pond: NSMT-P 149440 (1), 4.0

cm SL, set net, 26 June 2024; NSMT-P 149445 (2), 3.2–4.7 cm SL, fish trap, 26 June 2024; NSMT-P 150596 (3), 3.6–4.0 cm SL, fish trap, 30 Oct. 2024; NSMT-P 152060 (20), 2.7–5.5 cm SL, fish trap, 11 Aug. 2021; NSMT-P 152061 (21), 3.1–4.8 cm SL, fish trap, 11 Aug. 2021; NSMT-P 152062 (14), 3.2–5.4 cm SL, fish trap, 11 Aug. 2021; NSMT-P 153815 (1), 3.1 cm SL, set net, 26 June 2024; NSMT-P 153818 (3), 3.1–4.0 cm SL, set net, 26 June 2024.

Remarks. *Pseudorasbora pumila* was originally distributed on eastern Honshu Island (Kawase and Hosoya, 2015), but easily hybridizes with *P. parva*, leading to habitat loss across Japan (Konishi and Takata, 2004). Hybrids are often characterized by an intermediate number of pored lateral line scales (hereafter pored scales). Takata and Konishi (2006) proposed a hypothesis explaining that *P. pumila* is replaced by *P. parva* shortly after invasion by the latter of the former's distributional range, despite infertility of the F1 generation. Coexistence of the two species, as well as fertile hybrids were also reported (Koga and Goto, 2005). Generally, *P. pumila* (adults 4–7 cm TL) is considered distinguishable from *P. parva* (4–8 cm TL) by the former's incomplete lateral line (Hosoya, 2025).

Individual *P. pumila* inhabiting Fukiage Ohike Pond are descendants of individuals from Fukushima Prefecture, northern Honshu Island, which were stocked in the pond as a trial for *ex situ* preservation of the species in 1995. Fukiage Ohike Pond underwent the forementioned extensive repairs in 1993. Since the water was dried up completely at that time, no individuals of *P. parva* could survive in this pond, if present (none recorded in 1986, see Shinohara *et al*. 2026). However, *P. parva* was collected and detected by metabarcoding analyses in the pond during the present survey (Tables 1–3).

The number of pored scales most frequently ranged from 2 to 5 in the most anterior position (Hosoya, 2025), previous studies reporting the range as 2–6 (Miyadi, 1929), 0–5 (Sato and Kobayashi, 1954; Sugiyama, 1997), 3–6 (Hikita and Terao, 1959; Hikita, 1964, and 2–5 (Nakamura, 1963). Combining the data suggested a range of 0–6, although 2 specimens collected from natural habitats in the 1930's (currently in NSMT fish collection) were found with 7 pored scales, suggesting a greater range of 0–7. Specimens from Fukiage Ohike Pond had 0–27 (total) pored scales (Fig. 7A), most also having 0–6 anteriormost pored scales, though one individual had lateral line scale counts of 26 (left side) and 27 (right side), expressed as 26/27 or 26–27 (Fig. 7A). In *P. parva* collected from the moats and ponds in the Imperial Palace Inner Garden, the number of pored scales increased with growth, and the lateral line was completed (36–39 scales, see Hosoya, 2013, 2025) at ca. 60 mm TL (Fig. 7B).

One specimen (NSMT-P 153486, 52.7 mm TL) with 26/27 pored scales was identified as *P. parva* due to 50–55 mm-TL individuals possessing 22–33 pored scales. A second specimen with 9/9 anteriormost pored scales (NSMT-P 149441, 42.4 mm TL) was also determined to be *P. parva*, compared with 40–45 mm-TL specimens in Fig. 7B.

On the other hand, the largest specimen collected (NSMT-P 150595, 57. 1 mm TL, see Appendix II) had 9/9 anteriormost pored scales (Fig. 7A, and could be identified as either *P. parva* (33–36 pored scales) or *P. pumila* (0–7).

The two species and their hybrids could not be separated from each other below 35 mm TL (Fig. 7BC). Eight of 23 individuals 35–40 mm TL were identified as *P. pumila*, having 0–2 anteriormost pored-scale patterns (0/0, 0/1, 1/0, 1/1, 2/1, 2/1 and 2/2); and 14 of 24 specimens (40–45 mm TL) and 4 of 7 (55–60 mm TL) were identified as *P. parva* (Fig. 7A). Two of the largest specimens with 1–2 pored scales (55–60 mm TL) could not be identified.

Cobitidae

Misgurnus anguillicaudatus (Cantor, 1842)
[Japanese name: Dojo]
(Fig. 8A)

Collected from three waterbodies; detected in five (Table 5). Hasuike-bori Moat represented a new collection locality for this species.

Specimens. *n* = 14, 3.3–9.5 cm SL.

Kami-dokan-bori Moat (*n* = 9): NSMT-P 150597 (1), 7.0 cm SL, hand net, 30 Oct. 2024; NSMT-P 150598 (1), 10.2 cm SL, hand net, 30 Oct. 2024;

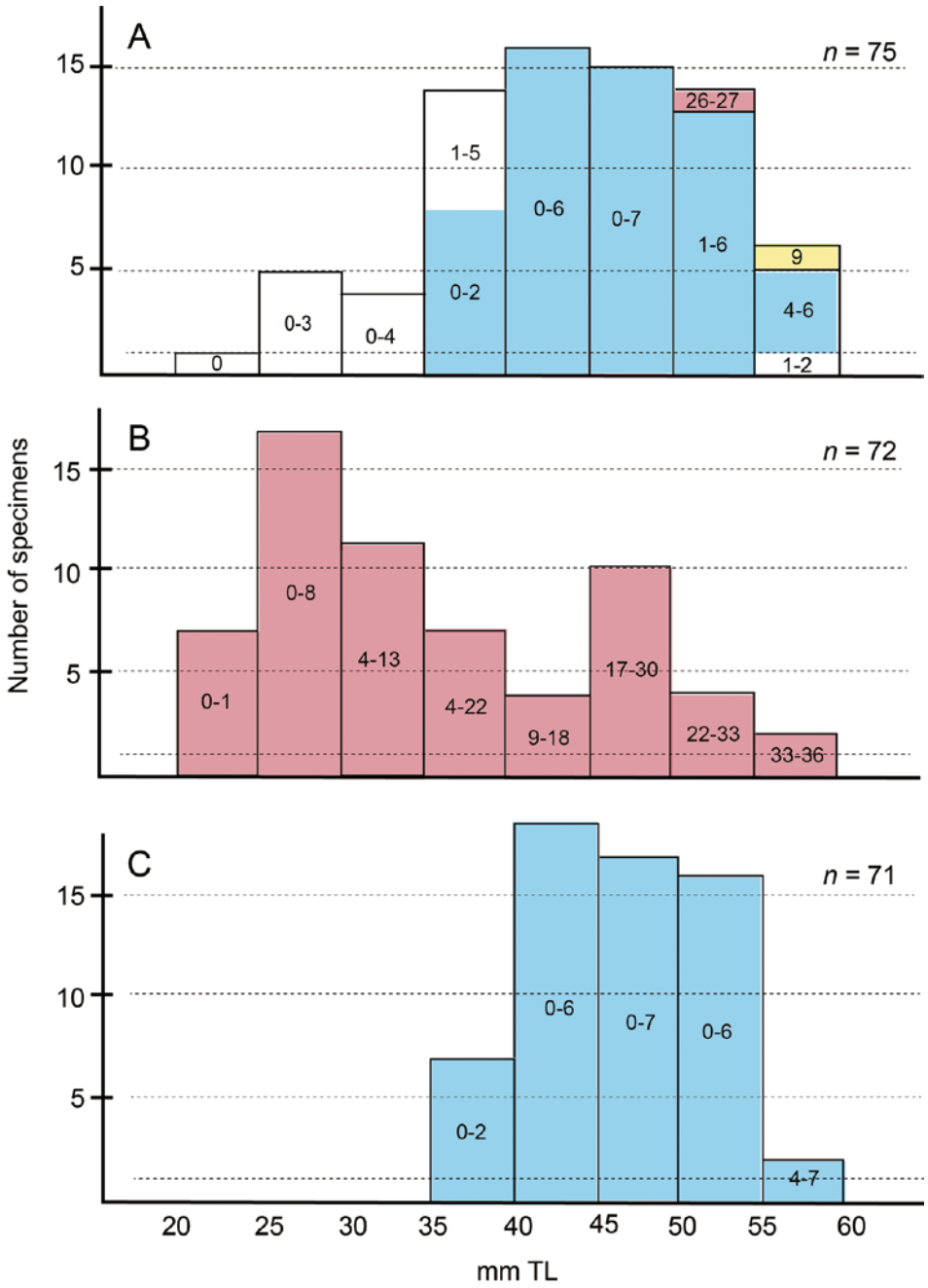

Fig. 7. Relationship between total length and number of pored lateral line scales in *Pseudorasbora* specimens. A, *Pseudorasbora* spp. (Fukiage Ohike Pond); B, *P. parva* (Naka-dokan-bori and Shimo-dokan-bori moats and Ninomaru-ike Pond); C, *P. pumila* (outside Imperial Palace). Numerals on bars indicate range of pored lateral line scale counts. White bars indicate *Pseudorasbora* sp. indet.; blue, *P. pumila*; red, *P. parva*; yellow, probable hybrid (see Appendices II–III for specimens).

NSMT-P 150601 (1), 9.1 cm SL, hand net, 30 Oct. 2024; NSMT-P 150602 (1), 9.0 cm SL, hand net, 30 Oct. 2024; NSMT-P 150603 (1), 5.5 cm SL, hand net, 30 Oct. 2024; NSMT-P 150604 (1), 5.6 cm SL, hand net, 30 Oct. 2024; NSMT-P 150605 (1), 7.3 cm SL, hand net, 30 Oct. 2024; NSMT-P 150606 (1), 6.5 cm SL, hand net, 30 Oct. 2024; NSMT-P 150607 (1), 5.7 cm SL, hand net, 30 Oct. 2024.

Shimo-dokan-bori Moat (*n* = 1): NSMT-P 148933 (1), 9.5 cm SL, fish trap, 22 May 2024.

Hasuike-bori Moat (*n* = 4): NSMT-P 149351 (1), 4.9 cm SL, hand net, 10 June 2024; NSMT-P 149352 (1), 3.5 cm SL, hand net, 10 June 2024; NSMT-P 149353 (1), 3.5 cm SL, hand net, 10 June 2024; NSMT-P 149354 (1), 3.3 cm SL, hand net, 10 June 2024.

Remarks. The metabarcoding analyses suggested both native and non-native lineages inhabiting waters in the Imperial Palace (Tables 2–4), although the former was only collected from the Shimo-dokan-bori and Hasuike-bori moats during this period.

Shinohara *et al*. (2026) reported two native-form specimens collected in Shimo-dokan-bori Moat on 21 March 1987, and Matsuura *et al*. (2000) collected eight specimens from Kami-dokan-bori Moat.

Siluriformes
Siluridae

Silurus asotus Linnaeus, 1758
[Japanese name: Namazu]
(Fig. 8B)

Collected from two waterbodies; detected in four (Table 5).

Specimens. *n* = 4, 28.4–39.9 cm SL.

Hirakawa-bori Moat (*n* = 1): NSMT-P 148991 (1), 39.9 cm SL, hand net, 24 May 2024.

Tenjin-bori Moat (*n* = 3): NSMT-P 150579 (1), 28.4 cm SL, fish trap, 10 Oct. 2024; NSMT-P 151006 (1), 38.8 cm SL, hook & line, 21 May 2024; NSMT-P 151007 (1), 37.5 cm SL, hook & line, 22 May 2024.

Remarks. Shinohara *et al*. (2026) noted the Report of the Imperial Household Agency's Garden Division in 1986, which stated "one individual was collected from Tenjin-bori Moat. Although there was information that it was visually observed in Hirakawa-bori Moat, it did not appear during the survey period." Matsuura *et al*. (2000) observed a single individual (approximately 30 cm TL) in Shimo-dokan-bori Moat, and Shimazu *et al*. (2000) collected two individuals (48–50 cm SL) from Hasuike-bori Moat and confirmed their tapeworm infection.

The metabarcoding analysis suggested that *Silurus* individuals existed in the Shimo-dokan-bori, Inui-bori, Hirakawa-bori and Tenjin-bori moats, suggesting a dynamic distribution via inter-moat movements.

Cyprinodontiformes
Poeciliidae

Gambusia affinis (Baird and Girard, 1853)
[Japanese name: Kadayashi]
(Fig. 8C)

Collected from nine waterbodies; detected in nine (Table 5).

Specimens. *n* = 787, 1.0–4.6 cm SL.

Naka-dokan-bori Moat (*n* = 108): NSMT-P 149362 (2), 1.3–1.3 cm SL, hand net, 10 June 2024; NSMT-P 149364 (6), 1.5–3.6 cm SL, hand net, 10 June 2024; NSMT-P 149374 (1), 3.6 cm SL, hand net, 10 June 2024; NSMT-P 149377 (1), 1.2 cm SL, hand net, 10 June 2024; NSMT-P 149381 (1), 3.5 cm SL, fish trap, 10 June 2024; NSMT-P 149584 (70), 1.9–4.0 cm SL, set net, 10 June 2024; NSMT-P 150585 (1), 2.7 cm SL, fish trap, 30 Oct. 2024; NSMT-P 150587 (24), 1.6–3.5 cm SL, hand net, 30 Oct. 2024; NSMT-P 150592 (2), 1.7–1.9 cm SL, hand net, 30 Oct. 2024.

Shimo-dokan-bori Moat (*n* = 5): NSMT-P 148931 (1), 3.3 cm SL, hand net, 22 May 2024; NSMT-P 148932 (1), 3.4 cm SL, hand net, 22 May 2024; NSMT-P 148967 (2), 3.0–3.1 cm SL, hand net, 22 May 2024; NSMT-P 150652 (1), 2.1 cm SL, hand net, 6 Nov. 2024.

Hasuike-bori Moat (*n* = 142): NSMT-P 149355 (4), 1.4–3.3 cm SL, hand net, 10 June 2024; NSMT-P 149453 (2), 1.7–2.0 cm SL, fish trap, 26 June 2024; NSMT-P 149454 (5), 1.8–3.5 cm SL, fish trap, 26 June 2024; NSMT-P 149457 (12), 1.3–3.6 cm SL, fish trap, 26 June 2024; NSMT-P 149458 (7), 1.8–3.1 cm SL,

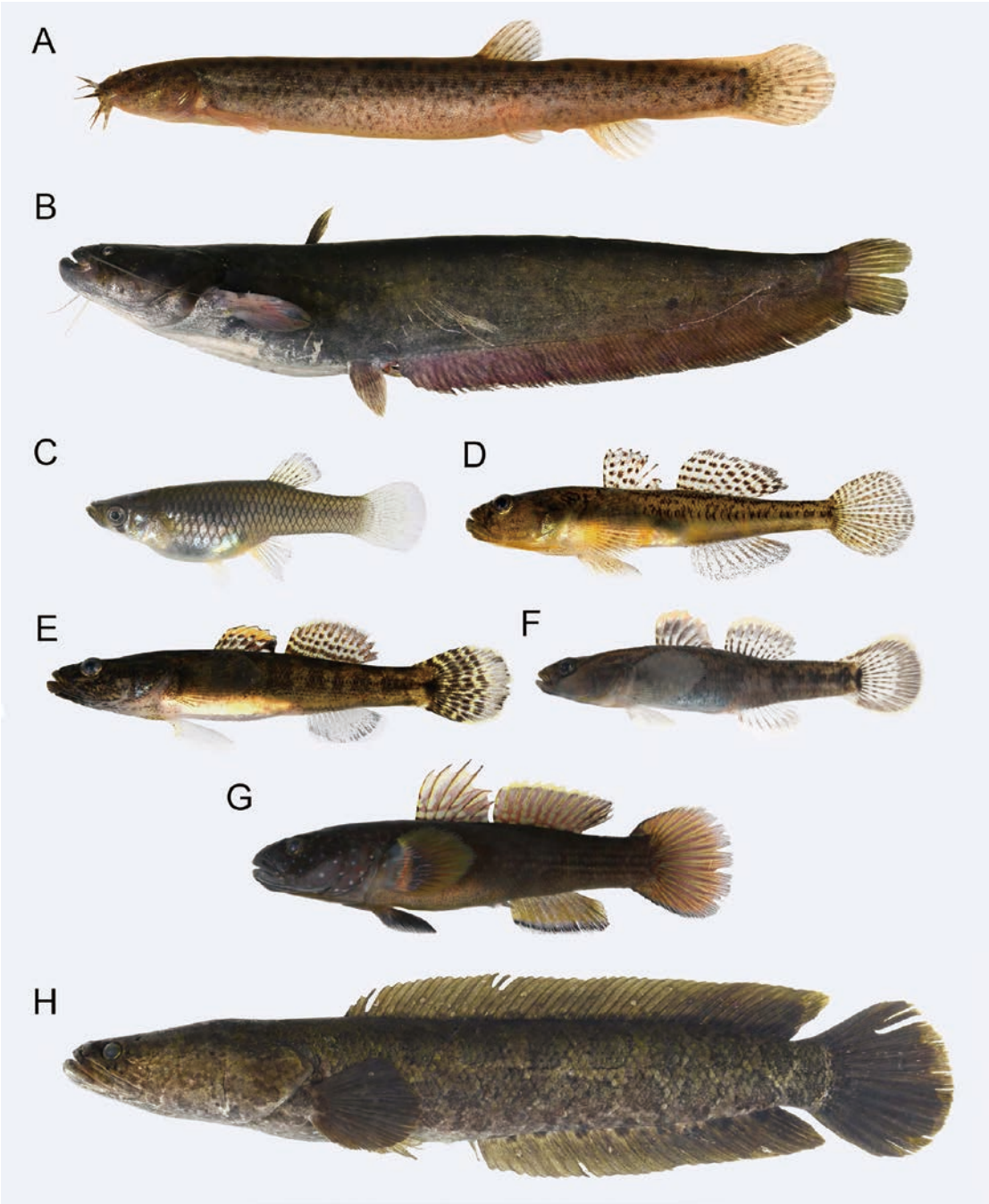

Fig. 8. Fresh specimens collected from Imperial Palace Inner Garden, Tokyo, Japan in 2024–2025. A, *Misgurnus anguillicaudatus*, native lineage, NSMT-P 148933, 95.0 mm SL, Shimo-dokan-bori Moat; B, *Silurus asotus*, NSMT-P 148991, 399.0 mm SL; C, *Gambusia affinis*, NSMT-P 148989, 29.7 mm SL, Hirakawa-bori Moat; D, *Gymnogobius castaneus*, NSMT-P 148939, 44.3 mm SL, Shimo-dokan-bori Moat; E, *Gymnogobius urotaenia*, NSMT-P 148937, 55.7 mm SL, Shimo-dokan-bori Moat; F, *Rhinogobius kurodai*, NSMT-P 148964, 35.8 mm SL, Shimo-dokan-bori Moat; G, *Tridentiger brevispinis*, NSMT-P 148981, 57.0 mm SL, Hirakawa-bori Moat; H, *Channa argus*, NSMT-P 148992, 412.0 mm SL, Hirakawa-bori Moat.

set net, 26 June 2024; NSMT-P 149463 (7), 1.8–2.3 cm SL, hand net, 26 June 2024; NSMT-P 149466 (9), 1.3–2.1 cm SL, hand net, 26 June 2024; NSMT-P 150639 (87), 1.5–3.8 cm SL, set net, 6 Nov. 2024; NSMT-P 150641 (4), 2.2–4.0 cm SL, hand net, 6 Nov. 2024; NSMT-P 150645 (1), 3.7 cm SL, hand net, 6 Nov. 2024; NSMT-P 150646 (4), 2.1–2.6 cm SL, fish trap, 6 Nov. 2024.

Inui-bori Moat ($n = 2$): NSMT-P 149069 (1), 2.2 cm SL, hand net, 24 May 2024; NSMT-P 150573 (1), 1.9 cm SL, fish trap, 28 Oct. 2024.

Hirakawa-bori Moat ($n = 6$): NSMT-P 148989 (1), 3.0 cm SL, hand net, 24 May 2024; NSMT-P 148990 (1), 2.9 cm SL, hand net, 24 May 2024; NSMT-P 150624 (4), 2.4–2.4 cm SL, hand net, 1 Nov. 2024.

Tenjin-bori Moat ($n = 42$): NSMT-P 149338 (1), 2.0 cm SL, hand net, 27 May 2024; NSMT-P 150617 (41), 2.1–3.2 cm SL, hand net, 1 Nov. 2024.

Ninomaru-ike Pond ($n = 23$): NSMT-P 149318 (9), 2.0–3.3 cm SL, fish trap, 27 May 2024; NSMT-P 149326 (6), 1.9–2.3 cm SL, hand net, 27 May 2024; NSMT-P 150574 (8), 1.8–2.8 cm SL, hand net, 28 Oct. 2024.

Fukiage Ohike Pond ($n = 138$): NSMT-P 149442 (1), 1.8 cm SL, set net, 26 June 2024; NSMT-P 149443 (1), 4.0 cm SL, hand net, 26 June 2024; NSMT-P 149444 (29), 1.0–3.7 cm SL, hand net, 26 June 2024; NSMT-P 149446 (23), 1.4–2.8 cm SL, hand net, 26 June 2024; NSMT-P 149447 (65), 1.0–2.7 cm SL, hand net, 26 June 2024; NSMT-P 149450 (2), 2.1–2.1 cm SL, set net, 26 June 2024; NSMT-P 150593 (1), 2.2 cm SL, fish trap, 30 Oct. 2024; NSMT-P 150594 (2), 2.3–2.4 cm SL, fish trap, 30 Oct. 2024; NSMT-P 152504 (5), 1.8–2.3 cm SL, fish trap, 11 Aug. 2021; NSMT-P 152505 (5), 1.7–2.7 cm SL, fish trap, 11 Aug. 2021; NSMT-P 152506 (4), 2.1–2.3 cm SL, fish trap, 13 Oct. 2021.

Kaintei Pond ($n = 323$): NSMT-P 149579 (323), 1.7–3.6 cm SL, hand net, 10 June 2024.

Remarks. In February 2006, *G. affinis* was designated as a specified alien species by a Cabinet Order based on the Invasive Alien Species Act, under the jurisdiction of the Ministry of the Environment. The species is included in the list "100 of the World's Worst Invasive Alien Species" (IUCN: International Union for Conservation of Nature and Natural Resources), all of which have made significant impacts on ecosystems and human activities.

All of the collected individuals were sacrificed and fixed for specimens because the import, transport and keeping are prohibited by the Invasive Alien Species Act (above). Although Matsuura *et al*. (2000) did not report any specimens from the Naka-dokan-bori and Shimo-dokan-bori moats, a total of 147 individuals had been collected from both moats and Hasuike-bori Moat in 1986 (Shinohara *et al*., 2026). Among the "inside" inner moats of the Imperial Palace, no collection records of *G. affinis* exist for Hakucho-bori Moat, a waterbody isolated from other areas and not subject to invasion. The absence of eDNA evidence supported such absence.

Perciformes
Gobiidae

Gymnogobius castaneus (O'Shaughnessy, 1875)
[Japanese name: Juzukakehaze]
(Fig. 8D)

Collected from six waterbodies; detected in seven (Table 5).

Specimens. $n = 220$, 1.2–4.7 cm SL.

Naka-dokan-bori Moat ($n = 96$): NSMT-P 149054 (44), 2.0–2.8 cm SL, fish trap, 24 May 2024; NSMT-P 149363 (2), 2.2–2.4 cm SL, hand net, 10 June 2024; NSMT-P 149365 (2), 2.3–2.7 cm SL, hand net, 10 June 2024; NSMT-P 149368 (8), 2.7–4.0 cm SL, fish trap, 10 June 2024; NSMT-P 149375 (9), 2.4–4.0 cm SL, hand net, 10 June 2024; NSMT-P 149383 (3), 2.6–2.8 cm SL, fish trap, 10 June 2024; NSMT-P 149586 (27), 2.2–3.7 cm SL, set net, 10 June 2024; NSMT-P 150588 (1), 3.9 cm SL, hand net, 30 Oct. 2024.

Shimo-dokan-bori Moat ($n = 176$): NSMT-P 148938 (1), 4.1 cm SL, fish trap, 22 May 2024; NSMT-P 148939 (1), 4.4 cm SL, fish trap, 22 May 2024; NSMT-P 148940 (1), 3.9 cm SL, fish trap, 22 May 2024; NSMT-P 148941 (1), 4.4 cm SL, fish trap, 22 May 2024; NSMT-P 148942 (1), 2.6 cm SL, fish trap, 22 May 2024; NSMT-P 148943 (1), 2.5 cm SL, fish trap, 22 May 2024; NSMT-P 148944 (1), 2.2 cm SL, fish trap, 22 May 2024; NSMT-P 148953 (1), 3.0 cm SL, set net, 22 May 2024; NSMT-P 148954

(3), 2.6–2.8 cm SL, set net, 22 May 2024; NSMT-P 148957 (6), 2.8–2.8 cm SL, hand net, 22 May 2024; NSMT-P 148958 (1), 2.9 cm SL, set net, 22 May 2024; NSMT-P 148959 (2), 2.6–2.9 cm SL, fish trap, 22 May 2024; NSMT-P 148962 (10), 1.2–2.6 cm SL, casting net, 22 May 2024; NSMT-P 148964 (5), 2.6–2.7 cm SL, hand net, 22 May 2024; NSMT-P 148965 (2), 2.4–2.7 cm SL, hand net, 22 May 2024; NSMT-P 148966 (1), 3.9 cm SL, hand net, 22 May 2024; NSMT-P 148971 (2), 1.8–2.1 cm SL, fish trap, 22 May 2024; NSMT-P 148972 (31), 2.4–4.0 cm SL, set net, 22 May 2024; NSMT-P 149039 (1), 4.7 cm SL, set net, 24 May 2024; NSMT-P 149040 (6), 2.3–4.6 cm SL, set net, 24 May 2024; NSMT-P 149043 (2), 3.9–4.6 cm SL, set net, 24 May 2024; NSMT-P 149051 (2), 2.2–2.6 cm SL, fish trap, 24 May 2024; NSMT-P 149053 (2), 2.4–2.5 cm SL, fish trap, 24 May 2024; NSMT-P 149058 (22), 2.4–4.4 cm SL, set net, 24 May 2024; NSMT-P 149062 (52), 2.5–4.4 cm SL, set net, 24 May 2024; NSMT-P 149065 (1), 2.3 cm SL, fish trap, 24 May 2024; NSMT-P 149066 (2), 2.4–2.7 cm SL, fish trap, 24 May 2024; NSMT-P 149360 (1), 4.1 cm SL, hand net, 10 June 2024; NSMT-P 150651 (5), 3.6–3.7 cm SL, hand net, 6 Nov. 2024; NSMT-P 150654 (4), 3.7–4.1 cm SL, hand net, 6 Nov. 2024; NSMT-P 150663 (5), 3.4–3.9 cm SL, hand net, 6 Nov. 2024.

Hasuike-bori Moat ($n = 23$): NSMT-P 149356 (4), 2.1–3.9 cm SL, hand net, 10 June 2024; NSMT-P 149460 (4), 2.3–2.9 cm SL, set net, 26 June 2024; NSMT-P 149461 (1), 3.1 cm SL, hand net, 26 June 2024; NSMT-P 149467 (11), 1.8–2.6 cm SL, hand net, 26 June 2024; NSMT-P 150640 (3), 3.6–3.7 cm SL, set net, 6 Nov. 2024.

Inui-bori Moat ($n = 32$): NSMT-P 149045 (1), 4.7 cm SL, set net, 24 May 2024; NSMT-P 149046 (10), 2.4–2.7 cm SL, set net, 24 May 2024; NSMT-P 149070 (1), 4.1 cm SL, hand net, 24 May 2024; NSMT-P 149335 (5), 1.7–1.9 cm SL, fish trap, 27 May 2024; NSMT-P 149581 (15), 1.9–4.5 cm SL, set net, 24 May 2024.

Hirakawa-bori Moat ($n = 5$): NSMT-P 149050 (2), 2.1–2.6 cm SL, casting net, 24 May 2024; NSMT-P 149331 (2), 2.7–2.7 cm SL, set net, 27 May 2024; NSMT-P 149587 (1), 1.8 cm SL, set net, 27 May 2024.

Tenjin-bori Moat ($n = 9$): NSMT-P 149339 (4), 2.5–2.9 cm SL, hand net, 27 May 2024; NSMT-P 150611 (1), 3.0 cm SL, set net, 1 Nov. 2024; NSMT-P 150616 (4), 2.9–3.2 cm SL, hand net, 1 Nov. 2024.

Remarks. Identification followed Stevenson (2002). Shinohara *et al*. (2026) noted the Report of the Garden Division in 1986, which stated "a total of 780 individuals were collected from Naka-dokan-bori, Shimo-dokan-bori, Hasuike-bori, Inui-bori, Hirakawa-bori, and Tenjin-bori moats. They primarily inhabit spring water and surrounding waterbodies." The present survey suggested that a favorable environment for the species existed within the study area; it was most abundant in Shimo-dokan-bori Moat (Table 1), where spring bubbles were observed sporadically from the moat floor during the survey.

In the 1986 survey (Shinohara *et al*., 2026) a total of 780 individuals were recorded from the Naka-dokan-bori, Shimo-dokan-bori, Hasuike-bori, Inui-bori, Hirakawa-bori and Tenjin-bori moats. The metabarcoding analysis detected the species at all collection sites, plus Fukiage Ohike Pond.

Gymnogobius urotaenia Hilgendorf, 1879
[Japanese name: Ukigori]
(Fig. 8E)

Collected from seven waterbodies; detected in same (Table 5).

Specimens. $n = 75$, 2.1–7.2 cm SL.

Naka-dokan-bori Moat ($n = 7$): NSMT-P 149369 (3), 3.3–3.8 cm SL, fish trap, 10 June 2024; NSMT-P 149376 (1), 3.7 cm SL, hand net, 10 June 2024; NSMT-P 149382 (2), 3.3–3.4 cm SL, fish trap, 10 June 2024; NSMT-P 150589 (1), 7.2 cm SL, hand net, 30 Oct. 2024.

Shimo-dokan-bori Moat ($n = 41$): NSMT-P 148937 (1), 5.6 cm SL, fish trap, 22 May 2024; NSMT-P 148948 (1), 5.8 cm SL, fish trap, 22 May 2024; NSMT-P 148949 (1), 3.5 cm SL, set net, 22 May 2024; NSMT-P 148952 (1), 3.3 cm SL, fish trap, 22 May 2024; NSMT-P 148955 (1), 3.6 cm SL, hand net, 22 May 2024; NSMT-P 148956 (2), 2.9–3.1 cm SL, hand net, 22 May 2024; NSMT-P 148960 (1), 3.5 cm SL, fish trap, 22 May 2024; NSMT-P 148961 (1), 8.1 cm SL, casting net, 22 May 2024; NSMT-P 148963 (1), 3.2 cm SL, hand net, 22 May 2024; NSMT-P 148970

(2), 2.3–3.3 cm SL, fish trap, 22 May 2024; NSMT-P 148973 (1), 3.5 cm SL, set net, 22 May 2024; NSMT-P 149037 (1), 5.6 cm SL, set net, 24 May 2024; NSMT-P 149038 (7), 2.7–5.2 cm SL, set net, 24 May 2024; NSMT-P 149052 (1), 3.1 cm SL, fish trap, 24 May 2024; NSMT-P 149059 (10), 2.9–3.5 cm SL, set net, 24 May 2024; NSMT-P 149063 (6), 2.7–3.6 cm SL, set net, 24 May 2024; NSMT-P 150656 (2), 5.1–5.4 cm SL, hand net, 6 Nov. 2024; NSMT-P 150657 (1), 7.3 cm SL, hook & line, 6 Nov. 2024.

Hasuike-bori Moat (*n* = 2): NSMT-P 149462 (2), 4.3–5.0 cm SL, hand net, 26 June 2024.

Inui-bori Moat (*n* = 2): NSMT-P 149047 (2), 3.2–5.2 cm SL, set net, 24 May 2024.

Hirakawa-bori Moat (*n* = 9): NSMT-P 148986 (6), 2.8–3.4 cm SL, hand net, 24 May 2024; NSMT-P 149049 (2), 2.4–2.5 cm SL, casting net, 24 May 2024; NSMT-P 150621 (1), 3.5 cm SL, fish trap, 1 Nov. 2024.

Tenjin-bori Moat (*n* = 7): NSMT-P 149340 (4), 2.2–3.3 cm SL, hand net, 27 May 2024; NSMT-P 149439 (3), 2.1–2.5 cm SL, fish trap, 31 May 2024.

Hakucho-bori Moat (*n* = 7): NSMT-P 149312 (1), 2.4 cm SL, set net, 27 May 2024; NSMT-P 149317 (1), 2.6 cm SL, hand net, 27 May 2024; NSMT-P 150565 (1), 7.7 cm SL, fish trap, 28 Oct. 2024; NSMT-P 150566 (4), 4.2–7.2 cm SL, fish trap, 28 Oct. 2024.

Remarks. Only two individuals were recorded from Hirakawa-bori Moat in the previous surveys in 1986 (Shinohara *et al*., 2026).

The metabarcoding analysis detected all collection sites.

Rhinogobius kurodai (Tanaka, 1908)
[Japanese name: Kurodahaze]
(Fig. 8F)

Collected from ten waterbodies, detected in nine (Table 5); eDNA sampling not conducted in Fukiage Benten-ike Pond.

Specimens. *n* = 65; 2.3–4.5 cm SL.

Kami-dokan-bori Moat (*n* = 2): NSMT-P 150599 (1), 4.1 cm SL, hand net, 30 Oct. 2024; NSMT-P 150600 (1), 2.3 cm SL, hand net, 30 Oct. 2024.

Naka-dokan-bori Moat (*n* = 3): NSMT-P 149055 (1), 3.1 cm SL, fish trap, 24 May 2024; NSMT-P 150590 (1), 3.6 cm SL, hand net, 30 Oct. 2024; NSMT-P 150591 (1), 3.1 cm SL, hand net, 30 Oct. 2024.

Shimo-dokan-bori Moat (*n* = 10): NSMT-P 148945 (1), 3.9 cm SL, hand net, 22 May 2024; NSMT-P 148946 (1), 3.6 cm SL, hand net, 22 May 2024; NSMT-P 149041 (1), 3.6 cm SL, set net, 24 May 2024; NSMT-P 149042 (2), 3.7–3.9 cm SL, set net, 24 May 2024; NSMT-P 149044 (1), 3.9 cm SL, set net, 24 May 2024; NSMT-P 149061 (1), 3.5 cm SL, set net, 24 May 2024; NSMT-P 149064 (1), 3.0 cm SL, set net, 24 May 2024; NSMT-P 150653 (2), 3.1–4.5 cm SL, hand net, 6 Nov. 2024.

Hasuike-bori Moat (*n* = 2): NSMT-P 150642 (1), 3.3 cm SL, hand net, 6 Nov. 2024; NSMT-P 150644 (1), 3.5 cm SL, hand net, 6 Nov. 2024.

Inui-bori Moat (*n* = 7): NSMT-P 149071 (3), 2.7–3.4 cm SL, hand net, 24 May 2024; NSMT-P 149333 (2), 2.6–2.6 cm SL, fish trap, 27 May 2024; NSMT-P 149582 (2), 2.7–3.5 cm SL, set net, 24 May 2024.

Hirakawa-bori Moat (*n* = 13): NSMT-P 148987 (2), 2.9–2.9 cm SL, hand net, 24 May 2024; NSMT-P 149057 (8), 2.4–3.2 cm SL, hand net, 24 May 2024; NSMT-P 149330 (2), 2.6–2.8 cm SL, set net, 27 May 2024; NSMT-P 150633 (1), 2.3 cm SL, set net, 1 Nov. 2024.

Tenjin-bori Moat (*n* = 4): NSMT-P 150612 (1), 2.3 cm SL, fish trap, 1 Nov. 2024; NSMT-P 150613 (3), 2.4–4.0 cm SL, hand net, 1 Nov. 2024.

Hakucho-bori Moat (*n* = 8): NSMT-P 149314 (8), 2.8–4.2 cm SL, hand net, 27 May 2024.

Ninomaru-ike Pond (*n* = 6): NSMT-P 149319 (3), 2.5–3.9 cm SL, fish trap, 27 May 2024; NSMT-P 149327 (3), 2.2–2.3 cm SL, hand net, 27 May 2024.

Kanbakutei Nagare Stream (*n* = 4): NSMT-P 149345 (1), 4.1 cm SL, hand net, 10 June 2024; NSMT-P 149346 (1), 3.9 cm SL, hand net, 10 June 2024; NSMT-P 149347 (2), 3.3–3.9 cm SL, hand net, 10 June 2024.

Benten-ike Pond (*n* = 6): NSMT-P 149348 (1), 3.6 cm SL, hand net, 10 June 2024; NSMT-P 149349 (1), 4.1 cm SL, hand net, 10 June 2024; NSMT-P 149350 (4), 3.3–4.2 cm SL, hand net, 10 June 2024.

Remarks. Identification followed Suzuki *et al*. (2017). *Rhinogobius kurodai* is distributed in certain areas of Tokyo and Kanagawa prefectures, but further detail

distribution requires thorough investigation in the future (Suzuki *et al*., 2017; Hosoya, 2025). Specimens were collected in all eight moats and three small waterbodies (excluding two artificial ponds which had dried up in the past, according to a BLIP memo). Since *R. kurodai* has not seen as a fisheries target or a desirable pet trade species, its origin of distribution here can be date back to long before (e.g., to the Edo Era) with least transferal.

Tridentiger brevispinis Katsuyama, Arai and Nakamura, 1972
[Japanese name: Numachichibu]
(Fig. 8G)

Collected from two waterbodies; detected in four (Table 5)

Specimens. *n* = 5, 3.5–5.7 cm SL.

Hirakawa-bori Moat (*n* = 4): NSMT-P 148981 (1), 5.7 cm SL, set net, 24 May 2024; NSMT-P 148982 (1), 3.5 cm SL, hand net, 24 May 2024; NSMT-P 149332 (2), 3.0–3.5 cm SL, set net, 27 May 2024.

Tenjin-bori Moat (*n* = 1): NSMT-P 153607 (1), 5.7 cm SL, hook & line, 18 July 2025.

Remarks. This species has not been reported in previous surveys of the "inside" inner moats. It usually occurs in middle and upper stream catchments, and is often landlocked in ponds or lakes (Hosoya, 2025). Although specimens were collected only from the Hirakawa-bori and Tenjin-bori moats, the metabarcoding analysis detected eDNA at both collection sites, as well as Kami-dokan-bori and Inui-bori moats. Significantly, Hirakawa-bori and Tenjin-bori moats are connected by a waterway, with the former also connected to the "outside" inner moats by overflow in times of excessive rainfall (see Figs. 1, 3 and 10). The species has been observed recently in the "outside" inner moats (Kokyogaien National Garden Office, 2022); also collected in the Ohte-bori and Shimizu-bori moats (adjacent to Hirakawa-bori Moat) in 2023 (Kokyogaien National Garden Office, personal communication). *Tridentiger brevispinis* is amphidromous, exhibiting upstream migration in its juvenile stage (Prince Akihito, 1987), so there is a possibility that its individuals eventually immigrated from the "outside" inner moat to Hirakawa-bori Moat in flooded seasons.

Channidae

Channa argus (Cantor, 1842)
[Japanese name: Kamuruchi]
(Fig. 8H)

Collected from four waterbodies; detected in five (Table 5).

Specimens. *n* = 6, 41.2–54.3 cm SL.

Hasuike-bori Moat (*n* = 1): NSMT-P 150649 (1), 46.3 cm SL, set net, 6 Nov. 2024.

Inui-bori Moat (*n* = 1): NSMT-P 150567 (1), 44.2 cm SL, trammel net, 28 Oct. 2024.

Hirakawabori Moat (*n* = 2): NSMT-P 148992 (1), 41.2 cm SL, casting net, 24 May 2024; NSMT-P 150625 (1), 54.3 cm SL, trammel net, 1 Nov. 2024.

Tenjin-bori Moat (*n* = 2): NSMT-P 151008 (1), 41.3 cm SL, hook & line, 9 July 2024; NSMT-P 151009 (1), 44.5 cm SL, hook & line, 10 July 2024.

Remarks. The means by which this species occurred in the Imperial Palace Inner Garden water systems is unclear (Shinohara *et al*., 2026), no specimens having been reported from Tenjin-bori Moat in the previous survey. Because Tenjin-bori and Hirakawa-bori moats are interconnected by a wide water duct, the shared occurrence was unsurprising.

The metabarcoding analysis detected the species throughout all of the collection sites.

Species unrecorded but potential inhabitants of the Imperial Palace Inner Garden Water Systems

Anguilliformes
Anguillidae

Anguilla japonica Temminck and Schlegel, 1847
[Japanese name: Nihon-unagi]

The metabarcoding analyses detected this species from the adjoined Hirakawa-bori and Tenjin-bori moats (Tables 2–3), the former being connected to

the "outside" inner moats via overflow in times of excessive rainfall (see Figs. 1, 3 and 10). Eels have been observed recently in the "outside" inner moats (Kokyogaien National Garden Office, 2022) and also collected in the Ohte-bori and Shimizu-bori moats (adjacent to Hirakawa-bori Moat) in 2023 (Kokyogaien National Garden Office, personal communication), so there is a possibility that eel individuals eventually immigrated from the "outside" inner moat in flooded season.

During the survey period, attempts to collect *A. japonica* were made using eel traps filled with chicken, fish and earthworm baits in the above two moats, but were unsuccessful. However, an Imperial Guard officer stationed at a police box on the western point of Hirakawa-bori Moat recorded sighting an (unidentified) eel near the surface (see "a" point, in Fig. 3).

Misgurnus chipisaniensis Shedko and Vasl'eva, 2022
[Japanese name: Kita-dojo]

The metabarcoding analyses indicated this species in the Naka-dokan-bori, Shimo-dokan-bori and Hasuike-bori moats (Tables 2–3). *Misgurnus chipisaniensis* was originally described from Sakhalin Island specimens (Shedko and Vasil'eva, 2022), the Japanese name having been established by Nakajima and Uchida (2017) for *Misgurnus* sp. (Clade A). Molecular phylogenetic trees in Shedko and Vasil'eva (2022) indicated that *M. chipisaniensis* formed a monophyletic group with part of *Misgurnus* spp. from Hokkaido and Honshu Islands. Hosoya (2025) considered *Misgurnus* sp. (Clade A) to be conspecific with *M. chipisaniensis*, despite having pointed out that the Japanese population should be compared with *Misgurnus buphoensis* Kim and Park, 1995 (North Korea) and *Misgurnus nikolskyi* Vasil'eva, 2001 (Amur River).

Agreement between Collected/Observed Fauna and Detected Fauna

Because some eDNA fragments corresponded to multiple species (see Appendix IV), strict one-to-one matching between eDNA and collection/observation results is not desirable, However, eDNA of collected/observed species could be interpreted as "detected" (given as "e" in Table 5).

Based on the faunal data (presence/absence) of fishes collected/observed and detected (Table 5), rates of agreement between the former and detected fauna for the respective waterbodies (on the 15 species collected or observed) were as follows: Kami-dokan-bori Moat 60%, Naka-dokan-bori Moat 80%, Shimo-dokan-bori Moat 93%, Hasuike-bori Moat 100%, Inui-bori Moat 73%, Hirakawa-bori Moat 87%, Tenjin-bori Moat 93%, Hakucho-bori Moat 100%, Ninomaru-ike Pond 100%, and Fukiage Ohike Pond 93%. The average was 88%.

Similarity Patterns of Species Occurrence and Waterbody Characteristics

PCA results are summarized in Fig. 9. In the first analysis, similarity/dissimilarity of occurrence tendency among fish species, PC1 and PC2 contributed to explain 45.3 % of the total multivariate variation (Fig. 9A). Along PC1, fish species occurring only in specific waterbodies were plotted on the positive side (*Pseudorasbora pumila* and *Oryzias latipes*), and those occurring in many waterbodies, on the negative side (*Rhinogobius kurodai* and *Pseudorasbora parva*). Along PC2, fishes inhabiting waterbodies with a certain level of internal water movement were plotted on the negative side (*Gymnogobius castaneus* and *Ctenopharyngodon idellus*), and those occurring in waterbodies with less internal water movement, on the positive side (*Misgurnus anguillicaudatus* and *R. kurodai*).

In the second analysis, similarity/dissimilarity of fauna among 13 waterbodies and three survey periods (1986, 2000 and 2026), PC1' and PC2' contributed to explain 41.8 % of the total multivariate variation (Fig. 9B). Along PC1', waterbodies with many fish species were plotted on the positive side (Tenjin-bori and Hirakawa-bori moats), and those with fewer species, on the negative side (Kaintei Pond and Fukiage Benten-ike Pond in 2024–2025). Along PC2', waterbodies with more pelagic fishes were plotted on the positive side (Tenjin-bori Moat in 2024–2025), and

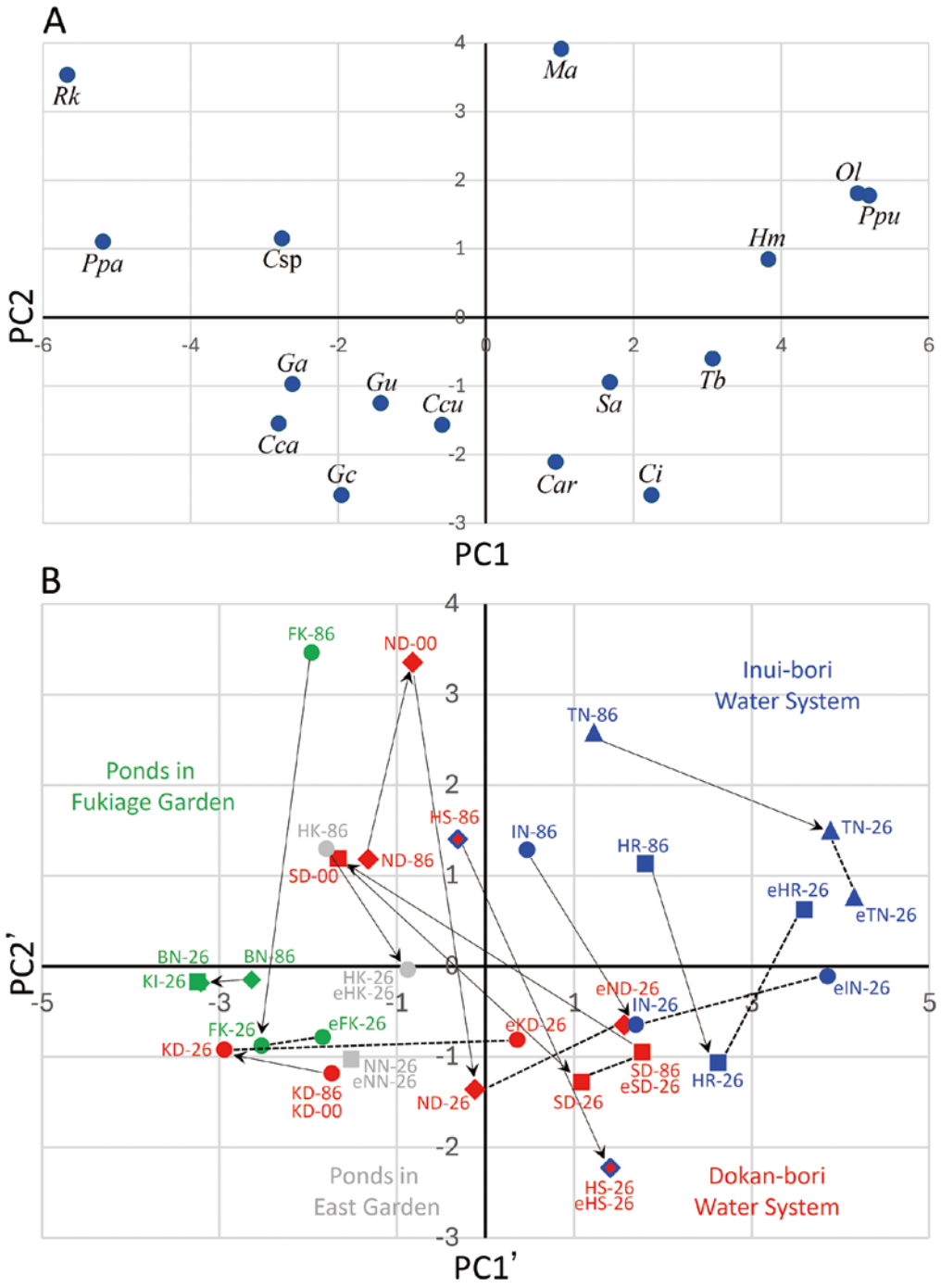

Fig. 9. PCA results for changing fish fauna of moats and ponds in Imperial Palace Inner Garden over 40 year period. Upper panel (A), fish species plotted on PC1 & PC2 plane (upper case, genus; lower case, species). Lower panel (B), survey waterbodies plotted on PC1' & PC2' plane reflecting time-series changes. "AA-##" indicates fish fauna of waterbody AA (abbreviations for waterbodies shown in Table 1) reported in given year (1986, 2000 and 2026). "e" indicates eDNA data.

those with fewer pelagic fishes, on the negative side (Hasuike-bori Moat in 2024–2025).

Considering faunal shifts in respective waterbodies, four geographical groups were recognized: ponds in the Fukiage Garden (green; Fukiage Ohike Pond, Kaintei Pond and Fukiage Benten-ike Pond, together with Kanbakutei Nagare Stream); Ponds in the East Garden (grey; Ninomaru-ike Pond and Hakucho-bori Moat); the Dokan-bori Water System (red; Kami-dokan-bori, Naka-dokan-bori, Shimo-dokan-bori and Hasuike-bori moats); and the Inui-bori Water System (blue; Inui-bori, Hirakawa-bori, and Tenjin-bori moats) (see Fig. 9). Each occupied specific spaces in the PC1'& PC2' plane, and shifted over time within their respective spaces. The waterbodies with many species (plotted on mid to right side) tended to shift in a right-down direction, indicating species numbers increased due to concentrated collection efforts and wide-ranging eDNA sampling (to right) and decreased pelagic environment (downward). The ranges of faunal shifts over time were greatest in the moats of the Dokan-bori Water System.

General Discussion

Species-level comparisons with previous surveys

Rhinogobius kurodai was collected in all eight moats and three small waterbodies. An endemic species to the Kanto Region, inhabiting small stagnant waterbodies, its wide distribution encompassing the eight moats (now with fewer inter-connections, see Fig. 10) indicated past inter-connections among the "inside" inner moats. In fact, the eight moats were inter-connected by continuous water flow according to a map of Edo Castle ca. 1608 (Tokyo Metropolitan Library, 2011–), which included Hakucho-bori Moat, now completely isolated. This suggested that the moats were constructed in series by introducing water from the adjacent moat to each newly constructed moat in turn. On the other hand, *Tridentiger brevispinis*, an amphidromous species which migrates upstream as juveniles (Prince Akihito, 1987), was collected from the "inside" inner moats for the first time. This record indicates recent partial inter-connection between the "inside" and "outside" inner moats. At the same time, no Largemouth Bass *Micropterus salmoides* or Bluegill Sunfish *Lepomis macrochirus* were collected/observed or detected. Both species were designated as Invasive Alien Species in 2005 (based on the Invasive Alien Species Act) due to their invasiveness, and have long been targets for extermination so as to conserve indigenous species. Their absence indicates that the rarity of overflow occasions between Hirakawa-bori Moat and "outside" inner moats has the effect of a "semi-connecting" pathway, retaining the opportunity for eventual migration, but preventing invasion of highly invasive alien species. Clearly, the presence/absence of fish species reflects the connectivity status among moats in the Imperial Palace.

Judging from the concentrated collecting efforts and eDNA analyses of the present survey, *Oryzias latipes* appears to have disappeared from the "inside" inner moats and ponds of the Imperial Palace. The species was reported as common in Naka-dokan-bori Moat, and was also collected from Shimo-dokan-bori Moat and Fukiage Ohike Pond in 1985–1999 (see Matsuura *et al*., 2000; Shinohara *et al*., 2026; the population in Fukiage Ohike Pond may have disappeared after extensive repair work in 1993, when the pond dried up completely). The Imperial Household Agency's Garden Division report in 1986 (see Shinohara *et al*., 2026 for details) included an alarming forecast that *Gambusia affinis* would compete against *O. latipes*, suppressing individual numbers of the latter. In fact, *G. affinis* has expanded its distribution throughout most of the "inside" inner moats and ponds, and is especially abundant in Hasuike-bori and Naka-dokan-bori moats (Table 1). Therefore, the decrease in numbers or disappearance of *O. latipes* is considered to have resulted from the invasion of *G. affinis*. On the other hand, individual numbers of the former in Shimo-dokan-bori, Inui-bori and Hirakawa-bori moats are suppressed, implying the existence of some key factors (relating to water flow status etc. discussed in the later section) suppressing the species in some moats, but not in Naka-dokan-bori and Hasuike-bori moats.

Carassius sp. "Ginbuna," an abundant species commonly collected in Kami-dokan-bori Moat during 1997–1999 (Matsuura *et al*., 2000), was not seen or detected in 2024–2025. The surveys in spring and autumn 2024 showed that the water volume in Kami-dokan-bori Moat had decreased compared to

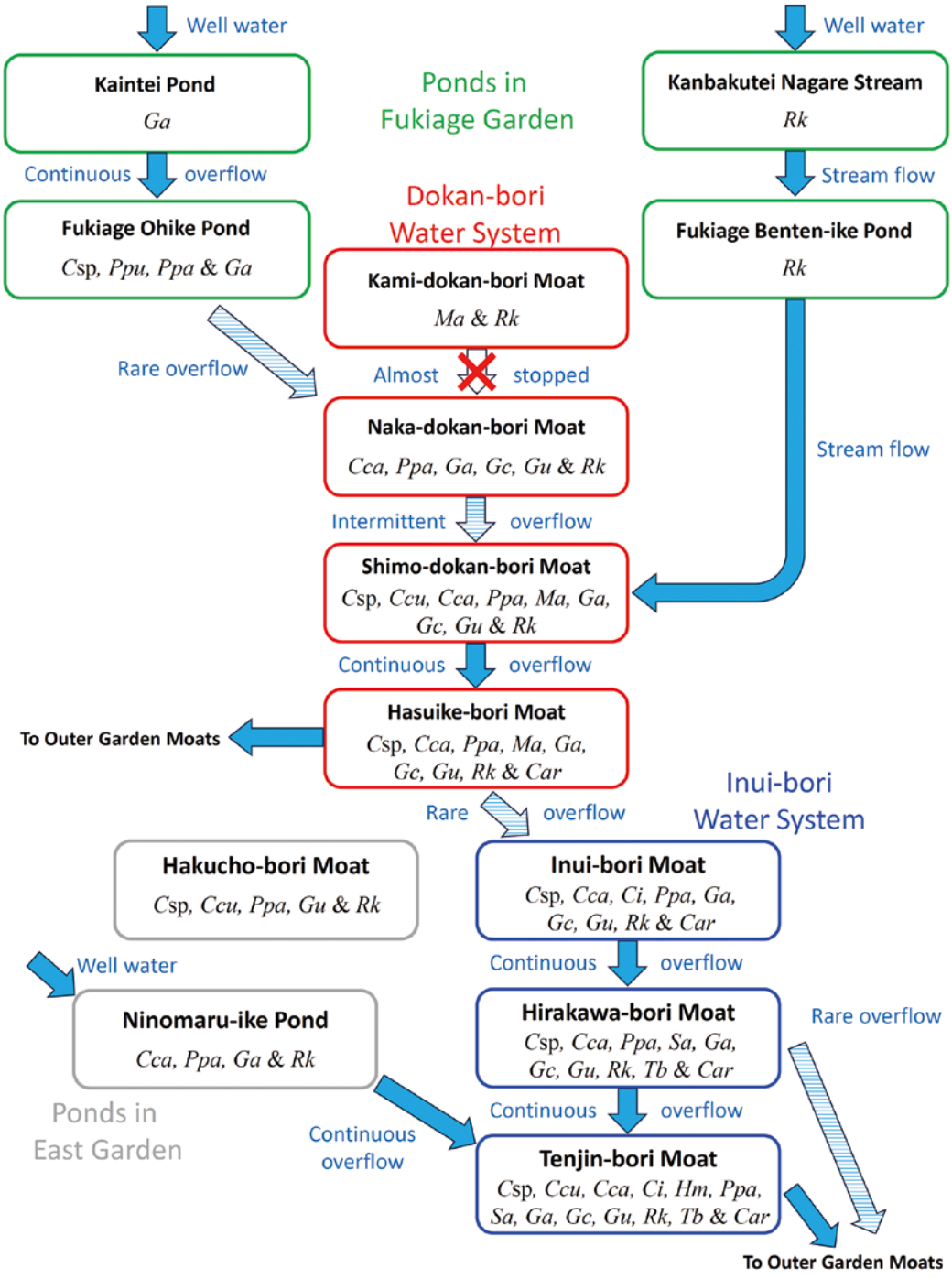

Fig. 10. Recent status of water flow among moats and ponds in Imperial Palace Inner Garden, showing relationships with species composition and water systems (Fig. 9). Abbreviations of species names as in Fig. 9.

1999, resulting in multiple fragmented waterbodies. Furthermore, the summer 2025 survey revealed worsening drought conditions, with the waterbody shrinking even further, with water temperature exceeding 30 degrees Celsius on one occasion. Not only was the soft muddy bottom exposed, but also suitable locations for fish traps could not be found. Following periods of rain, no fishes were observed in the resulting rainwater puddles. The decline or disappearance of *Carassius* sp. may be a result of reduced water volume in the moat due to construction works in/around the Palace, and/or extremes of climate warming in recent years.

Pseudorasbora parva is widely distributed in the water systems of the Imperial Palace Inner Garden, including Fukiage Ohike Pond. *P. pumila* was introduced to the pond in 1995 (after completion of the forementioned extensive repair work with its water dried up in 1993) as a voluntary trial of *ex situ* preservation of an endangered species. *Pseudorasbora pumila* easily hybridizes with *P. parva*, which has led to habitat loss of the former across Japan. The survey revealed the coexistence of both species (as well as a probable hybrid individual), a rare example of maintained coexistence without a rapid decrease in numbers of *P. pumila* (see Koga and Goto, 2005). The population status of the two species should be further studied in detail, with a view to future monitoring and appropriate management.

Fauna-level comparison with previous surveys

According to the PCA results on the fish faunal data (presence/absence) for waterbodies in the Imperial Palace Inner Garden, the fish species composition differed among four geographical areas: small ponds and a stream with a low number of species in the Fukiage Garden; a small pond and moat with a slightly higher number of species in the East Garden; large moats with an intermediate number of species, belonging to the Dokan-bori Water System; and large moats with high number of species, belonging to the Inui-bori Water System (Table 5, Figs. 1, 9). Among them, moats of the Dokan-bori Water System showed the largest range of faunal shifts across time (Fig. 9B), i.e., less stable. The large faunal shift in Fukiage Ohike Pond from 1986 to 2026 resulted from artificial activities including the repair work and transferal of fishes (see above). On the other hand, the large shifts among the moats of the Dokan-bori Water System occurred as natural changes, the greatest involving the fishes in Naka-dokan-bori Moat from 2000 to 2026, and the second, the fishes in Hasuike-bori Moat from 1986 to 2026, wherein large-sized pelagic fishes, such as *Carassius cuvieri, Ctenopharyngodon idellus* and *Hypophthalmichthys molitrix* disappeared, and benthic fishes such as *Misgurnus anguillicaudatus* and *Rhinogobius kurodai* newly appeared. Such faunal shifts suggested a change from a more to a less pelagic environment.

The Dokan-bori Water System was comparatively more fragmented with lesser water flow among the moats (Fig. 10). Kami-dokan-bori Moat has been separated from the downstream moats since the Meiji Era (Biological Laboratory of Imperial Household, 1989), and is already drying up considerably, aided by sedimentation. Naka-dokan-bori Moat has been semi-separated, losing continuous water input and with the inner water flow declining due to lush lotus vegetation covering most of the moat. Shimo-dokan-bori Moat retains considerable water and levels of water flow, but it has lost stable water input from Naka-dokan-bori Moat. Hasuike-bori, originally named for its lotus (hasu in Japanese) vegetation, now has declining water flow due to the lush lotus vegetation and is gradually drying up by the sedimentation, despite a certain level of water input. The lesser faunal shift in Kami-dokan-bori Moat may be attributable to a long precedence of the drying process. The fish species inhabiting the moats and ponds in the Imperial Palace Inner Garden respond to the level of internal water movement (Fig. 9A); therefore the latter, plus water flow among moats and relative volume of pelagic environment, are likely key factors for future monitoring, including further analysis of faunal data with quantity (individual numbers for respective species).

Utility of Environmental DNA

The metabarcoding analysis was effective for estimating fish fauna in the Imperial Palace, detecting species at all sites in which they had been collected and/or observed. It was also successful in detecting the potential distributions of *Anguilla japonica* and

Misgurnus chipisaniensis for the first time. In addition, the method suggested wider potential distributions of *Carassius* sp. "Ginbuna" (Kami-dokan-bori and Naka-dokan-bori moats), *Carassius cuvieri* (Naka-dokan-bori, Inui-bori and Hirakawa-bori moats), *Cyprinus carpio* (Kami-dokan-bori Moat), *Pseudorasbora parva* (Kami-dokan-bori Moat), *Misgurnus anguillicaudatus* (non-native from Inui-bori Moat; native from Tenjin-bori Moat), *Silurus asotus* (Shimo-dokan-bori and Inui-bori moats), *Gambusia affinis* (Kami-dokan-bori Moat), *Gymnogobius castaneus* (Fukiage Ohike Pond), *Tridentiger brevispinis* (Kami-dokan-bori and Inui-bori moats), and *Channa argus* (Naka-dokan-bori Moat). While such usefulness is acknowledged, considerable caution was necessary in interpreting the results. For example, some doubtful freshwater species were detected in Kami-dokan-bori Moat (isolated from other moats, its water supply comprising almost entirely rainfall and spring water). Because the moat is difficult to approach (surrounded by cliffs), *Cyprinus carpio* and *Hypophthalmichthys molitrix* (Cyprinidae), and *Poecilia reticulata* (Poeciliidae) are unlikely to have been released there. It is also an unlikely environment for *Oncorhynchus masou* subspp. (Salmonidae) and *Mugil cephalus cephalus* (Mugillidae). In addition, *Cottus* spp. (Cottidae) are not suited for Tenjin-bori Moat, requiring clear water and greater water flow. Such detections of eDNA may have originated from cross-contamination from the clothing or tools of field investigators. Given the proximity of the survey area to other waterbodies in the city, the impact of waterfowl is an additional challenge.

Water in Kami-dokan-bori Moat was suspected of other contaminants, other than marine fish DNA originating from the investigators. Such DNA elements were also detected in the Shimo-dokan-bori, Inui-bori and Hirakawa-bori moats (see Appendix V), many representing food consumption (Appendix V), suggestive of rainwater and wastewater discharge draining over land. Only marine fish DNA (11 species) was detected in a small clear water puddle in Kami-dokan-bori Moat (St. KD5 on 11 July 2025). Clearly, wild animals, such as crows, may also disperse waste contaminants.

Future monitoring methods

The eDNA metabarcoding analysis suggested the existence of *Anguilla japonica* in the Hirakawa-bori and Tenjin-bori moats, and *Misgurnus chipisaniensis* in Naka-dokan-bori Moat. Future collecting surveys are necessary to confirm these occurrences. Considering the results of the present survey, a future survey design combining collecting/observing/detecting methods should be appropriate, e.g., eDNA metabarcoding for faunal status as a whole (taking contamination problems into consideration), fish-census by visual observation for large evasive fish species, and specimen collections for dubious or suspicious species detected as eDNA, in addition to minimal collection of voucher specimens of commonly occurring species. For such method designing in future, we could collect various fundamental information from the survey this time.

Acknowledgments

We appreciate the various support of staff members of the Garden Division (Imperial Household Agency, IHA), special permissions to survey Fukiage Garden from the Board of Chamberlains (IHA), and cooperation of staff members to search and inspect Imperial Palace fish records in the Archives Division (Imperial Household Archives of IHA). Taro Kurima (former student of University of Tsukuba) helped during the field survey in 2024. Toshiaki Kuramochi (Meguro Parasitological Museum) donated fish specimens following parasitological research in 2021 and 2024. Kokyogaien National Garden Office (Ministry of the Environment) donated fish specimens collected for the alien fish survey project in 2024. Ryota Kawanishi (Hokkaido University of Education) and Mikhail V. Nazarkin (Zoological Institute, Russian Academy of Sciences) helped provide literature information. Seigo Kawase (Lake Biwa Museum) gave us information on *Pseudorasbora* distribution. Yutaro Inoue (National Museum of Nature and Science) helped to identify loaches. Special thanks go to Masatoshi Nakamura (IDEA Consultants Inc.) for valuable suggestions and explanation of metabarcoding analysis in his laboratory. We also thank Graham S. Hardy (New Zealand) for English proofreading.

References

Akihito, Prince, 1987. Chichibu-rui (*Tridentiger obscurus* and its closely related species). In: Mizuno, N. and Goto, A. (eds.): Freshwater Fishes in Japan—their Distribution, Variation & Speciation, pp. 167–178. Tokai University Press, Tokyo (in Japanese).

Atsumi, K., H. Y. Song, H. Senou, K. Inoue & K. Mabuchi, 2017. Morphological features of an endangered Japanese strain of *Cyprinus carpio*: reconstruction based on seven SNP markers. Journal of Fish Biology 90 (3): 936–953.

Biodiversity Center of Japan, 2021. A survey manual for conducting eDNA analysis on freshwater fish and amphibians, Version 2. Nature Conservation Bureau, Ministry of the Environment. *https://www.biodic.go.jp/edna/edna_top.html* (in Japanese). [Accessed on 27 October 2025].

Biological Laboratory of Imperial Household (ed.), 1989. Flora Sedis Imperatoris Japoniae. 546 pp. Hoikusha Publishing Co., Osaka (in Japanese with English abstract).

Fukuda, K., 1990. Studies on breeding long fin Nishikigoi as a new brood stock. Saitamaken. Suisan Shikenjo Kenkyu Hokoku 49: 53–61.

Ganzawa N., 1985. Maboroshi no kawa, Chidori-ga-fuchi gawa wo Ou: Toshin de Kawa wo Dou Oshietaka (Invisible Chidori-ga-fuchi River: How I Taught the Students about the Local River). Chigakukyoiku and Kagaku-undo 14: 23–29 (in Japanese).

Hikita, T., 1964. On the recent distribution of two small cyprinid fishes, *Pseudorasbora parva pumila* (Miyadi) and *P. parva parva* (Temmink and Schlegel) in Hokkaido Island, Japan. Scientific Reports of the Hokkaido Salmon Hatchery (18): 113–116.

Hikita, T. & T. Terao, 1959. Notes of a new locality and some morphological observations of a cyprinoid fish, *Pseudorasbora parva pumila* (Miyadi) in eastern Hokkaido. Scientific Reports of the Hokkaido Fish Hatchery (14): 67–71 (in Japanese).

Hosoya, K., 2013. Cyprinidae. In: Nakabo, T. (ed.) Fishes of Japan with Pictorial Keys to the Species, Third Edition, pp. 308–327, 1813–1819. Tokai University Press, Hadano (in Japanese).

Hosoya, K. (ed.), 2025. Freshwater fishes of Japan, Fourth Edition. 584 pp. Yama-kei, Chiyoda (in Japanese).

Kanai, T., 1981. Ohori wo Megutte (History of Moats). v + 99 pp. Kyogakusha, Chiyoda (in Japanese).

Kawase, S. & K. Hosoya, 2015. *Pseudorasbora pugnax*, a new species of minnow from Japan, and redescription of *P. pumila* (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters 25(4): 289–298.

Koga, K. & A. Goto, 2005. Genetic structures of allopatric and sympatric populations in *Pseudorasbora pumila pumila* and *Pseudorasbora parva*. Ichthyological Research 52: 243–250.

Kokyogaien National Garden Office (Ministry of the Environment), 2022. Kokyo no Ohori (The Moats of the Imperial Palace). Official leaflet. https://www.env.go.jp/garden/kokyonoohori.pdf [Accessed on 27 Oct. 2025].

Konishi, M. & K. Takata, 2004. Impact of asymmetrical hybridization followed by sterile F1 hybrids on species replacement in *Pseudorasbora*. Conservation Genetics 5: 463–474.

Kuramochi, T., A. Shinohara, H. Ono, S. Nomura, U. Jinbo, H. Saito, K. Hasegawa, I. Nishiumi, S. Kawada, M. Tomokuni, M. Owada & T. Kiyoshi, 2014. Flora and fauna of the Imperial Palace, Tokyo II. Fauna. Memoirs of the National Museum of Nature and Science (50): 1–7 (in Japanese).

Maeda, K., D. Nishimura, H. Maemura, K. Morishima, Q. Zhang, T. Umino, H. Nakagawa & K. Arai, 2003. Identification and distribution of gynogenetic clones in silver crucian carp *Carassius langsdorfii* collected from the Dokanbori moats of the Imperial Palace, Tokyo, Japan. Nippon Suisan Gakkaishi 69(2): 185–191 (in Japanese with English abstract).

Matsuura, K., G. Shinohara, K. Arai & M. Nishida, 2000. Fishes from the inside moats of the Imperial Palace, Tokyo, Japan. Memoirs of the National Science Museum (35): 57–68.

Miya, M., Y. Sato, T. Fukunaga, T. Sado, L. Y. Poulsen, K. Sato., T. Minamoto, S. Yamamoto, H. Yamanaka, H. Araki, M. Kondoh & W. Iwasaki,

2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. Royal Society Open Science 2:150088.

Miyadi, D., 1929. Notes on a new cyprinid fish, *Pseudorasbora pumila*, sp. nov. from Sinai-numa, Prov. Rikuzen. The Zoological Society of Japan 12: 445–448.

Nakabo, T. (ed.), 2013. Fishes of Japan with Pictorial Keys to the Species, Third Edition. l + 2431 pp. Tokai University Press, Hadano (in Japanese).

Nakajima, J. & R. Uchida, 2017. Loaches of Japan-Natural History and Culture. 224 pp. Yama-kei, Tokyo (in Japanese).

Nakamura, M., 1963. Keys to the Freshwater Fishes of Japan Fully Illustrated in Colors. 259 pp. Hokuryukan, Chiyoda (in Japanese).

NEWJEC Inc., 2023. Koukyo Seibutsusou Chousa Gyoumu Houokokusho (Survey Report on Imperial Palace Biota). 122 pp. (in Japanese).

Saruwatari, T., J. A. López & T. W. Pietsch, 1997. Cyanine Blue: a versatile and harmless stain for specimen observation. Copeia 1997 (4): 840–841.

Sato, S.-i. & K. Kobayashi, 1954. Notes on the ichthyofauna of the fresh western Hokkaido, Japan. Bulletin of the Faculty of Fisheries, Hokkaido University 4 (4): 268–272.

Shedko, S. V. & E. D. Vasil'eva, 2022. A new species of the pond loaches *Misgurnus* (Cobitidae) from the south of Sakhalin Island. Journal of Ichthyology 62 (3): 356–372.

Shimazu, T., T. Kuramochi, J. Araki & M. Machida, 2000. Digenean, cetode, and nematode parasites of freshwater fishes of the Imperial Palace, Tokyo. Memoirs of the National Science Museum (35): 211–231.

Shinohara, G., M. Hayashi & M. Kishida, 2026. Fish specimens and survey report in 1986 for moats and ponds in the Imperial Palace Inner Garden, Tokyo in the late 1980s. Bulletin of the National Museum of Nature and Science, Series A 52 (1): 73–81 (in Japanese).

Stevenson, D. E., 2002. Systematics and distribution of fishes of the Asian goby genera *Chaenogobius* and *Gymnogobius* (Osteichthyes: Perciformes: Gobiidae), with the description of a new species. Species Diversity 7 (3): 251–312.

Sugiyama, H., 1997. Tansuigyo Akita Tokuhon (Akita Tokuhon of Fresh Water Fishes). 185 pp. Mumyosha Shuppan, Akita (in Japanese).

Suzuki, M., 1975. Edo to Edojo (Edo and Edo Castle). 220 pp. Shin Jimbutsu Oraisha, Tokyo (in Japanese).

Suzuki, T., M. Aizawa & K. Shibukawa, 2017. *Rhinogobius kurodai* (Tanaka, 1908): its revised diagnosis particularly for distinguishing from *Rhinogobius* sp. BF, and a comment on an ill-defined congener, known as "*Rhinogobius* sp. OR morphotype Gi-toshoku" in Japan. Bulletin of Museum of Natural and Environmental History, Shizuoka (10): 57–66 (in Japanese with English abstract).

Suzuki, S., M. Tsuchiya, K. Watanebe & M. Tamagawa, 1958. Sogyo *Ctenopharyngodon idellus* (Cuvier et Valenciennes) no jinkosaibyo ni kansuru kenkyu–tsuketari, Hakuren *Hypophthalmichthys molitrix* (C. et V.) no jinkosaibyo ni tsuite (Research on artificial breeding of Grass carp *Ctenopharyngodon idellus* (Cuvier et Valenciennes) with appendix on artificial breeding of *Hypophthalmichthys molitrix* (C. et V.)). Saitamaken Suisanshikenjo Gyomu Hokoku, 9: 1–59 (in Japanese).

Takata, K. & M. Konishi, 2006. Shinai-motsugo no Hozen eno Mosaku—Naganoken no Shinai-motsugo wo Reini—(Exploring Conservation of Shinai-motsugo: a Case Study in Nagano Prefecture). Pages 109–116 In: Hosoya, K. and K. Takahashi (eds.) Burakkubasu wo Taijisuru-Shinai-motsugo Satonokai karano Messeji. Koseisha Koseikaku, Tokyo (in Japanese).

The eDNA Society, 2022. Environmental DNA Sampling and Experiment Manual Version 2.2. Otsu, Japan (in Japanese). https://ednasociety.org/wp-content/uploads/2022/06/eDNA_manual_ver2_2.pdf [Accessed on 27 Oct. 2025].

The eDNA Society, 2024. Environmental DNA Sampling and Experiment Manual Version 3.0. Otsu, Japan (in Japanese). https://ednasociety.org/wp-content/uploads/2024/08/%E7%92%B0%E5%A2%83DNA%E8%AA%BF%E6%9F%BB%E3%83%BB%E5%AE%9F%E9%A8%93%E3%83%9E%E3%83%8B%E3%83%A5%E3%82%A2

%E3%83%AB_ver3_0.pdf [Accessed on 27 Oct. 2025].

Tokyo Metropolitan Library, 2011–. Keichō Edo Ezu (Tokyo Shiryō Collection A11-1) in EDO TOKYO Digital Museum https://www.library.metro.tokyo.lg.jp/portals/0/edo/tokyo_library/english/database/index.html?ky=&and_or=0&ca=6&submit=Search [Accessed on 27 Oct. 2025].

Appendix I. Record of 2024–2025 survey fieldwork.

Waterbody names	Spring 2024		Autumn 2024		Summer 2025	
	Collection / observation	Water sampling	Collection / observation	Water sampling	Collection / observation	Water sampling
① Kami-dokan-bori Moat	22 May	22 May	30 Oct.	30 Oct.	7 July	7 & 11 July
② Naka-dokan-bori Moat	24 May, 10 June	22 May	30 Oct.	30 Oct.	11 July	—
③ Shimo-dokan-bori Moat	22 & 24 May, 10 June	22 May	30 Oct, 6 Nov.	6 Nov.	11 July	—
④ Hasuike-bori Moat	10 & 26 June	26 June	6 Nov.	6 Nov.	—	—
⑤ Inui-bori Moat	24 & 27 May	24 May	28 Oct.	28 Oct.	4 June	11 July
⑥ Hirakawa-bori Moat	24 & 27 May	24 May	1 Nov.	1 Nov.	11 & 18 July	11 July
⑦ Tenjin-bori Moat	27 & 31 May	27 May	1 Nov.	1 Nov.	11 & 18 July	11 July
⑧ Hakucho-bori Moat	27 May	27 May	28 Oct.	28 Oct.	—	—
⑨ Ninomaru-ike Pond	27 May	27 May	28 Oct.	28 Oct.	—	—
⑩ Fukiage Ohike Pond	26 June	26 June	30 Oct.	30 Oct.	—	—
⑪ Kaintei Pond	10 June	—	—	—	—	—
⑫ Kanbakutei Nagare Stream	10 June	—	—	—	—	—
⑬ Fukiage Benten-ike Pond	10 June	—	—	—	—	—

Appendix II. Unidentified specimens collected.

Carassius sp. indet.

Specimens. *n* = 18, 1.2–3.3 cm SL.

Shimo-dokan-bori Moat (*n* = 1): NSMT-P 149067 (1), 2.6 cm SL, set net, 24 May 2024.

Hasuike-bori Moat (*n* = 1) : NSMT-P 149465 (1), 3.2 cm SL, set net, 26 June 2024.

Inui-bori Moat (*n* = 12): NSMT-P 148976 (1), 2.9 cm SL, set net, 24 May 2024; NSMT-P 148977 (1), 2.6 cm SL set net , 24 May 2024; NSMT-P 148978 (1), 2.7 cm SL, set net, 24 May 2024; NSMT-P 148979 (1), 2.3 cm SL, set net, 24 May 2024; NSMT-P 148980 (1), 2.0 cm SL, set net, 24 May 2024; NSMT-P 149074 (6), 2.0–2.5 cm SL, hand net, 24 May 2024; NSMT-P 149334 (1), 2.4 cm SL, fish trap, 27 May 2024.

Hirakawa-bori Moat (*n* = 3): NSMT-P 148988 (1), 2.2 cm SL, hand net, 24 May 2024; NSMT-P 149048 (1), 2.7 cm SL, casting net, 24 May 2024; NSMT-P 149329 (1), 3.3 cm SL, set net, 27 May 2024.

Hakucho-bori Moat (*n* = 1): NSMT-P 149316 (1), 1.2 cm SL, hand net, 27 May 2024.

Pseudorasbora sp. indet.

Specimens. *n* = 55, 20.8–34.2 mm SL.

Fukiage Ohike Pond: NSMT-P 149448 (2), 20.8–22.4 mm SL, hand net, 26 June 2024; NSMT-P 149449 (27), 21.9–34.2 mm SL, set net, 26 June 2024; NSMT-P 152063 (4), 24.0–31.8 mm SL, fish trap, 13 Oct. 2021; NSMT-P 153810 (1), 29.3 mm SL, fish trap, 26 June 2024; NSMT-P 153811 (8), 23.1–31.8 mm SL, fish trap, 11 Aug. 2021; NSMT-P 153812 (2), 21.6–24.0 mm SL, fish trap, 11 Aug. 2021; NSMT-P 153813 (1), 23.3 mm SL, fish trap, 11 Aug. 2021. NSMT-P 153814 (1), 31.2 mm SL, fish trap, 30 Oct. 2024; NSMT-P 153819 (9), 20.2–32.7 mm SL, set net, 26 June 2024.

Probable hybrid (*Pseudorasbora parva* x *P. pumila*)

Specimen. *n* = 1.

Fukiage Ohike Pond: NSMT-P 150595 (1), 46.2 mm SL, fish trap, 30 Oct. 2024.

Appendix III. Comparative specimens used in Fig. 7BC.

Pseudorasbora parva

Specimens. *n* = 62, 14.4–51.9 mm SL.

Naka-dokan-bori Moat: NSMT-P 149370 (7), 24.7–38.8 mm SL, fish trap, 10 June 2024; NSMT-P 149585 (1 of 43), 38,3 mm SL, set net, 10 June 2024; NSMT-P 149379 (1), 34.8 mm SL, fish trap, 10 June 2024; NSMT-P 149361 (1), 17.4 mm SL, hand net, 10 June 2024; NSMT-P 149056 (18), 17.4–23.1 mm SL, fish trap, 24 May 2024.

Shimo-dokan-bori Moat: NSMT-P 148936(1), 51.9 mm SL, fish trap, 22 May 2024; NSMT-P 148951 (1), 38.9 mm SL, fish trap, 22 May 2024; NSMT-P 148935

Appendix IV. Reference species data for metabarcoding analyses (DDBJ at https://ddbj.nig.ac.jp/arsa/advanced_search?lang=en for accession no.)

Anguillidae	LC492306.1	*Anguilla japonica*	Nihon-unagi
Cyprinidae	LC795716.1 OR094955.1 LC795716.1	*Carassius* sp.	Ginbuna
ibid.	ibid.	*Carassius buergeri* subsp. 2	Kinbuna
ibid.	ibid.	*Carassius buergeri buergeri*	Ohkinbuna
ibid.	ibid.	*Carassius buergeri grandoculis*	Nigorobuna
ibid.	ibid.	*Carassius auratus*	Kingyo
ibid.	ibid.	*Carassius* sp.	(Ryukyu Islands)
Cyprinidae	MK291479.1 LC552361.1	*Cyprinus carpio*	Koi (reared form)
Cyprinidae	MK134852.1 LC552360.1	*Cyprinus carpio*	Koi (wild form)
Cyprinidae	LC468872.1 LC552362.1	*Carassius cuvieri*	Gengorobuna
Cyprinidae	MG827396.1	*Ctenopharyngodon idellus*	Sogyo
Cyprinidae	MF180233.1	*Hypophthalmichthys molitrix*	Hakuren
ibid.	ibid.	*Hypophthalmichthys nobilis*	Kokuren
Cyprinidae	LC193418.1 LC552387.1	*Pseudorasbora parva*	Motsugo
ibid.	ibid.	*Pseudorasbora interrupta*	(Mt. Fenghuang)
Cyprinidae	NC_008665.1	*Pseudorasbora pumila*	Shinai-motsugo
Cobitidae	LC492322.1	*Misgurnus anguillicaudatus*	Dojo (non-native)
ibid.	ibid.	*Misgurnus anguillicaudatus*	Dojo (native)
Cobitidae	LC069460.1	*Misgurnus chipisaniensis*	Kita-dojo
Siluridae	MN171302.1 LC458075.1	*Silurus asotus*	Namazu
ibid.	ibid.	*Silurus lithophilus*	Iwatoko-namazu
ibid.	ibid.	*Silurus tomodai*	Tanigawa-namazu
ibid.	ibid.	*Silurus soldatovi*	(Amur River)
Poeciliidae	PP059110.1, PP620812.1	*Gambusia affinis*	Kadayashi
Gobiidae	LC474233.1	*Gymnogobius urotaenia*	Ukigori
Gobiidae	LC519434.1	*Gymnogobius castaneus*	Juzukakehaze
ibid.	ibid.	*Gymnogobius taranetzi*	Shinjikohaze
ibid.	ibid.	*Gymnogobius* sp. 2	Hokuriku-juzukakehaze
Gobiidae	LC385178.1 LC835952.1 LC852806.1	*Rhinogobius kurodai*	Kurodahaze
ibid.	ibid.	*Rhinogobius* sp.	Tou-yoshinobori
ibid.	ibid.	*Rhinogobius brunneus*	Kuro-yoshinobori
ibid.	ibid.	*Rhinogobius fluviatilis*	Oh-yoshinobori
ibid.	ibid.	*Rhinogobius* sp. KZ	Kazusa-yoshinobori
ibid.	ibid.	*Rhinogobius* sp. OM	Ohmi-yoshinobori
ibid.	ibid.	*Rhinogobius tyoni*	Shimahire-yoshinobori
ibid.	ibid.	*Rhinogobius mizunoi*	Ruri-yoshinobori
Gobiidae	LC519443.1 LC552538.1	*Tridentiger obscurus*	Chichibu
ibid.	ibid.	*Tridentiger brevispinis*	Numa-chichibu
ibid.	ibid.	*Tridentiger kuroiwae*	Naganogori
Channidae	LC474256.1	*Channa argus*	Kamuruchi

(1), 42.9 mm SL, fish trap, 22 May 2024; NSMT-P 149036 (1 of 5), 38.5 mm SL, set net, 24 May 2024; NSMT-P 149060 (1 of 7) 43.4 mm SL, set net, 24 May 2024.

Hasuike-bori Moat: NSMT-P 149456 (5 of 13), 17.4–34.5 mm SL, fish trap, 26 June 2024; NSMT-P 149451 (1), 34.3 mm SL, fish trap, 26 June 2024; NSMT-P 149459 (2 of 24), 29.5–36.7 mm SL, set net, 26 June 2024.

Hakucho-bori Moat: NSMT-P 149315 (1 of 19), 26.8 mm SL, hand net, 27 May 2024.

Ninomaru-ike Pond: NSMT-P 149320 (14), 31.0–49.5 mm SL, fish trap, 27 May 2024.

Pseudorasbora pumila

Specimens. *n* = 62, 28.9–42.0 mm SL.

Iwate Prefecture: NSMT-P 76865 (1), 36.0 mm SL, Morioka, Ota Village, 15 June 1932; NSMT-P 76868 (1), 42.0 mm SL, Shiwa County, Katayose, 30 Aug. 1931.

Miyagi Prefecture: NSMT-P 76621 (1), 33.0 mm SL, Kurihara County, Izunuma, 13 Dec. 1931; NSMT-P 73890 (5), 31.0–34.0 mm SL, Tome County, Nitta Village, Naganuma, 16 Aug. 1931; NSMT-P 74049 (49 of 51), 30.0–47.1 mm SL, Tome County, Shinden Village, Naga-numa Pond, 16 Aug. 1931.

Nagano Prefecture: NSMT-P SK (=Sigenkagaku Kenkyusyo) 519 (4), 28.9–35.0 mm SL, Sugadaira-gawa River, 8 June 1941.

Appendix V. Species (accession no. / read no.) determined as false positives from metabarcoding analyses (DDBJ at https://ddbj.nig.ac.jp/arsa/advanced_search?lang=en for accession no.)

Kami-dokan-bori Moat

St. KD1 (22 May 2024)—Clupeidae: *Etrumeus micropus* (LC385202.1 / 3,222); Chlorophthalmidae: *Chlorophthalmus* (LC385145.1 / 415); Macrouridae: *Coelorinchus kishinouyei* (LC474166.1 / 817); Lophiidae: *Lophius litulon* (AB974498.1 / 6,389); Mugilidae: *Mugil cephalus cephalus* (LC492367.1 / 2,514); Poeciliidae: *Poecilia reticulata* (PP059110.1 / 2,605); Carangidae: *Seriola rivoliana* (AB517559.2 / 3,260) and *Trachurus japonicus* (LC519400.1 / 2,594); Uranoscopidae: *Xenocephalus elongatus* (LC506657.1 / 3,304); Gobiidae: *Rhinogobius* spp. (LC069780.1 / 801); Balistidae: *Abalistes filamentosus* (LC278276.1 / 10,119).

St. KD2 (22 May 2024)—Lophiidae: *Lophius litulon* (AB974498.1 / 3,315); Mugilidae *Mugil cephalus cephalus* (LC492367.1 / 7,435).

St. KD5 (7 July 2025)—Engraulidae: *Engraulis japonica* (LC519336.1 / 271).

St. KD9 (11 July 2025)—Exocoetidae: *Cheilopogon* spp. (LC852796.1 / 19); Sebastidae: *Sebastes babcocki* (OR641504.1 / 359); *Sebastes* spp. (OR641504.1 / 125,802); Scorpaenidae: *Scorpaena neglecta* (LC872038.1 / 108); Triglidae: *Chelidonichthys spinosus* (LC552437.1 / 6); Serranidae: *Niphon spinosus* (AB972249.1 / 59); Carangidae: *Seriola dumerili* (LC519399.1 / 11), *Trachurus japonicus* (LC552504.1 / 32); Lutjanidae: *Lutjanus ophuysenii* (LC829650.1 / 36); Haemulidae: *Parapristipoma trilineatum* (LC812904.1 / 8); Sparidae: *Evynnis tumifrons* (AB972235.1 / 20,611); Sciaenidae: *Pennahia argentata* (LC552454.1 / 64,224); Hexagrammidae: *Hexagrammos otakii* (AP006786.1 / 201).

St. KD10 (11 July 2025)—Salmonidae: *Oncorhynchus masou* subspp. (LC552427.1 / 39,964).

Shimo-dokan-bori Moat

St. SD2 (24 May 2024)—Engraulidae: *Engraulis japonica* (LC519336.1 / 72); Gonostomatidae: *Cyclothone atraria* (LC193322.1 / 4); Gadidae: *Gadus chalcogrammus* (MH035603.1 / 10).

Hasuike-bori Moat

St. HS3 (6 Nov. 2024)—Scombridae: *Katsuwonus pelamis* (OQ846187.1 / 75).

Inui-bori Moat

St. IN2 (24 May 2024)—Gobiidae: *Rhinogobius* spp. (LC716654.1 / 32).

Hirakawa-bori Moat

St. HR3(1 Nov. 2024)—Carangidae: *Seriola dumerili* (LC519399.1 /386).

Tenjin-bori Moat

St. TN5 (11 July 2025)—Cottidae: *Cottus pollux* or *Cottus* sp. medium-sized egg type (LC552496.1 / 28).

2024–2025年の調査で採集・観察された皇居内苑濠池の魚類

篠原現人・岸田宗範・中江雅典・藤原恭司・佐藤真央・林　公義

皇居の生物相調査の第Ⅲ期において，皇居内苑の濠や池で2024年春期・秋期と2025年夏期に魚類相調査を行った．魚類相調査は第Ⅰ期に行って以来，第Ⅱ期には実施しなかったため，四半世紀ぶりの調査である．第Ⅰ期は上道灌濠，中道灌濠，下道灌濠を対象にしたが，第Ⅲ期はこれらに加え，蓮池濠，乾濠，平川濠，天神濠，白鳥濠，二の丸池および吹上御苑内の小規模水域を対象とした．調査期間中に採集された証拠標本は，国立科学博物館に登録・保存された．今回は，採集調査と目視観察に加え，環境水中のDNAメタバーコーディング解析を行った．その結果，皇居内苑には少なくとも15種が生息することが確認された．採集調査では，皇居外苑濠からは記録されていたヌマチチブが初めて内苑濠（平川濠・天神濠）からも確認された．目視観察のみで確認された種は，ソウギョ（乾濠・天神濠）とハクレン（天神濠）であった．環境DNAにより生息が示唆されたニホンウナギ（平川濠・天神濠）とキタドジョウ（中道灌濠・下道灌濠）は，標本の採集はされなかった．採集調査・目視観察で確認された魚種に関する環境DNAの検出有無との一致率は，上道灌濠では60％と低めであったが，全水域平均では88％であった．吹上大池では，過去に系統保存の試みとしてシナイモツゴが放流されていたが，今回，採集標本および環境DNAにより生息が確認された．主として中道灌濠において1986–1999年に報告されていたメダカは，環境DNAも含め今回は確認できなかった．他方，メダカと競合するとされるカダヤシが，中道灌濠をはじめ多くの水域で多数採集された．1997–1999年に上道灌濠で多く採集されていたギンブナが，今期は発見されなかった．過去の調査と今回の調査の結果を合わせて主成分分析を行ったところ，皇居内苑のエリアごとに魚類相が異なり，その中でも道灌濠水系の魚類相の経年変化は相対的に大きいことが判明し，同水系で徐々に進行する止水化や一部陸地化の現象との関連性が示唆され，今後より詳細な分析が必要である．