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Full Papers

Japanese Journal of Ichthyology

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Distribution of native Japanese mtDNA haplotypes of the common carp (*Cyprinus carpio*) in Lake Biwa

Kohji Mabuchi, Hiroshi Senou, Hirohiko Takeshima, Katsuki Nakai and Mutsumi Nishida

Abstract A recent mitochondrial (mt) DNA survey revealed that the native Japanese population of the common carp has been endangered by the introduction of non-native domesticated strains: more than half of the haplotypes detected so far in Japanese waters originated from Eurasian strains. In expectation that Lake Biwa, the largest freshwater body in Japan, contains a relatively pure native population, we conducted a survey of mtDNA haplotypes in the lake, collecting 856 common carp from 40 localities in the lake. Of these, 606 specimens were caught by various nets (gill net, set net, net trap, etc.) used at depths from 1 to 5 m, 148 specimens were caught by a trawl net used at depths from 30–70 m, and 102 specimens that had washed ashore during mass mortality caused by Koi herpes virus (KHV) in 2004 were collected by hand. For each of the specimens, the mitochondria-encoded cytochrome *b* gene was genotyped (Japanese native or non-native haplotypes) by PCR using allele-specific primers. Analysis of haplotype frequencies showed that the native Japanese haplotype was significantly more frequent in deep off-shore waters and waters along the steep northern coast of the North Basin (about 80% on average), than in shallow coastal waters in the South Basin and waters along the eastern coast of the North Basin (less than 50% on average). These results indicate that the deep waters of the lake may contain a relatively pure native population that is of prime importance for conserving the native Japanese common carp. Among the results, it is notable that more than 90% of the KHV-killed specimens had native Japanese haplotypes.

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Genetic structure of natural populations of *Misgurnus anguillicaudatus* in Ehime Prefecture impacted by recent exotic introductions, inferred from mitochondrial DNA analysis

Takaaki Shimizu and Motohiro Takagi

Abstract The genetic structure of natural populations of *Misgurnus anguillicaudatus* in Ehime Prefecture and the impact of introduced specimens were investigated by partial mitochondrial DNA sequence analysis. A total of 452 specimens from 59 samples were analyzed, comprising 49 populations from eight Prefectures (including 38 from Ehime Prefecture), six populations from Korea, and four samples of food material and fish bait obtained in Ehime Prefecture. Fifty-one haplotypes were identified, falling into two large clades (B-1 and B-2; average nucleotide divergence: $4.2 \pm 0.6\%$), which formed a sister lineage of *Paramisgurnus dabrianus* (bootstrap value: 100%). Among clade B-1 members, the haplotypes from 25 populations representing 15 water systems in Ehime Prefecture revealed genetic divergence correlated with their geographic distribution (nucleotide divergence distance: 0.3–4.0%). The maximum parsimony network of these haplotypes suggested one major haplotype (89% of the clade's individuals in Ehime Prefecture) with genetically close satellites present in specific rivers or locations. Some divergent haplotypes, somewhat similar to haplotypes from other Prefectures or from Korea, may have resulted from artificial introductions into Ehime. In clade B-2, five of the 17 haplotypes were distributed in only four river systems in Ehime Prefecture, without any apparent relation to geographic features, and

showed genetic identity or closeness to haplotypes of imported and commercial (food material, fish bait) lines. These results are strongly indicative of recent artificial introductions of alien individuals into natural waters in Ehime Prefecture, resulting in serious genetic disturbances.

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Multiplex PCR-based genotyping of mitochondrial DNA from two species of ocean sunfish from the genus *Mola* (Tetraodontiformes: Molidae) found in Japanese waters

Yusuke Yamanoue, Kohji Mabuchi, Etsuro Sawai, Yoichi Sakai, Hiroaki Hashimoto and Mutsumi Nishida

Abstract Morphological identification of two ocean sunfish species (genus *Mola*) is not an easy task due to the giant body size and poor understanding of their intraspecific variation. A rapid, reliable method of identifying mitochondrial DNA from two *Mola* species in Japanese waters was designed for PCR-based genotyping of the mitochondrial control region. Two allele-specific primers were developed for the mitochondrial control region of each species: CRMolaAL and CRMolaBL for *Mola* spp. A and B, respectively. Using the same reverse primer (H884-12S), specific primers for *Mola* spp. A and B were designed to amplify ca. 400- and 500-bp fragments, respectively. A pair of fish universal primers (L1969-16S and H2582-16S) amplifying ca. 700 bp was used as an internal control. Multiplex PCR reactions including these five primers produced a species-specific fragment for either *Mola* sp. A or B with an internal control. This approach has major advantages over other molecular

species-identification methods in speed and cost-efficiency, and greatly helps in determining *Mola* species.

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Reproductive ecology and life history of Itasenpara bitterling *Acheilognathus longipinnis* in a conservation pond in Himi, Toyama, Japan

Jyun-ichi Kitamura and Masaki Nishio

Abstract The reproductive ecology and life history of the Itasenpara bitterling *Acheilognathus longipinnis* was investigated in a conservation pond in Himi, Toyama, Japan. Based on ovulation and ovipositor length in females, the breeding season of *A. longipinnis* was estimated as occurring in September and October, peaking in early October. Female *A. longipinnis* began to mature from ca. 34.1 mm SL. They had large clutch sizes (max 59 eggs), depositing the highly adhesive and comparatively large eggs (3.05 mm³, 3.47 mm major and 1.29 mm minor axis) by means of a short ovipositor (mean 16.2 mm). The embryos lived inside mussel gills until May the following year, at that time emerging as juveniles (9 mm SL) and growing to maturity (ca. 35 mm SL) over the next four months. About 800 individuals emerged from host mussels in May 2007. The adult population size was estimated to be 200 individuals in November 2007, comprising 1+ (120 fishes) and 2+ (80 fishes) age classes, ranging from 31.2–59.0 mm SL. The survival rate of *A. longipinnis* from May to November (after their emergence from host mussels) was about 15%, confirming the successful role of the conservation pond in maintaining the *A. longipinnis* population.

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**Spawning ecology and schemes for the conservation of an endangered cyprinid,
Pseudorasbora pumila subsp. sensu Nakamura (1969), including comparisons with
a related species, *Pseudorasbora parva***

Yuichi Kano, Jyun-ichi Kitamura and Kouichi Kawamura

Abstract The reproductive ecology of an endangered cyprinid, *Pseudorasbora pumila* subsp. sensu Nakamura (1969), was elucidated by examination of the spawning substrate. A field experiment indicated that oviposition in *P. pumila* subsp. was restricted to relatively large ($>191 \text{ cm}^3$) stones in shallower depths ($<37 \text{ cm}$). Another “choice” experiment showed that *P. pumila* subsp. oviposited almost always on the bricks, whereas a related species *Pseudorasbora parva* preferred the sticks. The bricks were sometimes occupied by *Procambarus clarkia*, and the oviposition of *P. pumila* subsp. was negatively correlated with the presence of *P. clarkia*. The reproductive behavior of *P. pumila* subsp. was recorded using a waterproof video camera, characteristic behavior including bottom digging, egg/substrate cleaning, ovipositing and egg eating being observed. Because the size and depth of stones play a vital role in reproduction of *P. pumila* subsp., conservation effort for this subspecies should include the shallow placement of large stones in suitable habitat.

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Short Reports

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Predation by *Zacco platypus*, *Tribolodon hakonensis* and *Gymnogobius petschiliensis* on Ayu (*Plecoglossus altivelis altivelis*) larvae under light and dark experimental conditions

Tetsuya Tsuruta, Ryoto Koike, Hirohiko Takeshima, Takaharu Natsumeda and Kei'ichiro Iguchi

Abstract This study aims to evaluate the predation pressure during daytime and nighttime on larvae of Ayu, *Plecoglossus altivelis*, by comparing the number consumed by three predatory fish species between light and dark experimental conditions. Four of five individuals for *Zacco platypus* consumed more Ayu larvae in the light condition than in the dark condition. The number of Ayu larvae consumed by all four individuals for *Tribolodon hakonensis* in the light condition was about twice that in the dark condition. For *Gymnogobius petschiliensis*, three of five individuals ate few Ayu larvae, one showed a higher and the other showed a lower feeding activity under the light condition. Because two out of three predators showed a consistently higher feeding activity on Ayu larvae under the light condition, we suggest that the diurnal predation pressure is a selective factor for not only nocturnal drift in Ayu larvae but also concentrating hatching after sunset.

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First record of *Phoxinus lagowskii steindachneri* established in southern Hokkaido, Japan

Fumihito Tashiro, Kunio Amaoka, Atsubumi Mikami and Mamoru Yabe

Abstract One hundred and fifty one specimens of the Amur minnow *Phoxinus lagowskii steindachneri* were collected from the An'noro River, Assabu, southern Hokkaido, Japan, from 2007 to 2009. Previously recorded primarily from eastern Honshu Island in Japan, the subspecies has hitherto not been recorded from southern Hokkaido. Its occurrence in the An'noro River is most likely to have resulted from artificial introductions, together with Ayu (*Plecoglossus altivelis altivelis*) from Lake Biwa, from the mid 1980's to 1995, since which time the former has become established.

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Breeding season in the Japan Sea and Pacific Ocean forms of threespine stickleback *Gasterosteus aculeatus* in the Bekanbeushi River system, eastern Hokkaido, Japan

Manabu Kume

Abstract Breeding seasons in the anadromous Japan Sea (JS) and Pacific Ocean (PO) populations of threespine stickleback *Gasterosteus aculeatus* were estimated from seasonal changes in gonad somatic indices (GSI), kidney somatic indices (KSI), male

nuptial coloration and female maturity. Seasonal changes in reproductive traits, except for male GSI, showed similar tendencies in each form. Mature females and PO males became apparent in May, increasing in numbers as the breeding season progressed until mid June, thereafter gradually decreasing and disappearing in July. Mature JS adults also became apparent in May, increasing in numbers as the breeding season progressed until mid June. The present and previous investigations both indicated that breeding season did not function to temporally isolate the two forms from each other.

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Redescription of a gobiid fish, *Stiphodon pelewensis* Herre, 1936, with comments on its standard Japanese name

Toshiyuki Suzuki, Jiro Sakaue and Hiroshi Senou

Abstract Four specimens of *Stiphodon pelewensis* Herre, 1936 (Perciformes: Gobiidae: Sicydiinae), collected from a freshwater stream on Babelthuap Island, Palau Islands, are redescribed, including hitherto unrecorded information on the cephalic lateral-line system and fresh coloration. Previous confusion of the species with *Sicyopus zosterophorum*, resulting in uncertainty of the status of common Japanese names, can be rectified by the adoption of Akabouzuhaze and Fudehaze as the standard Japanese names for *Sicyopus zosterophorum* and *Stiphodon pelewensis*, respectively.

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