Zoological Studies

Identification of Exotic Sailfin Catfish Species (*Pterygoplichthys*, Loricariidae) in Taiwan Based on Morphology and mtDNA Sequences

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Li-Wei Wu, Chien-Chin Liu, and Si-Min Lin (2011) Identification of exotic sailfin catfish species (*Pterygoplichthys*, Loricariidae) in Taiwan based on morphology and mtDNA sequences. *Zoological Studies* **50**(2): 235-246. The sailfin catfish, a collection of several morphologically similar species belonging to the genus *Pterygoplichthys* of the Loricariidae from South America, has been widely introduced to tropical and subtropical freshwater environments worldwide and has caused serious ecological impacts. Although listed as one of the most serious invasive species, the taxonomic status of this fish in Taiwan has never been precisely described. In this study, we used morphological traits and mitochondrial DNA sequences to identify the fish which currently occur in Taiwanese rivers. Evidence from both datasets rejected the existence of *P. multiradiatus*, which was once the most widely applied name in local references. *Pterygoplichthys gibbiceps* and *P. joselimaianus*, 2 other species commonly appearing in the aquarium fish trade, were also not detected in the wild. Our results indicated that the fish occurring in Taiwanese rivers should be identified as *P. pardalis* and *P. disjunctivus*. Nevertheless, fish collected from the wild present a contiguous variation, among which roughly 28.3% could not be defined as either species and thus were grouped as an intermediate form. Morphological identification did not match the 2 mitochondrial clades. Compared to a native population of a pure-line *P. pardalis* collected from the Amazon River at Manaus, Brazil, exotic populations showed much wider morphological variations and higher genetic diversities. We put forth 2 hypotheses and 1 speculation to explain the current situation: (1) *P. disjunctivus* might just be a synonym of *P. pardalis*; (2) the exotic populations may have originated from hybridization between the 2 species or allopatric populations; and (3) superiority of the hybrid may have helped increase their fitness during invasions. http://zoolstud.sinica.edu.tw/Journals/50.2/235.pdf

Key words: *Anolis*, Hybridization, Invasion, *Tamarix*, Tolerance.

Introduction of exotic species has caused numerous negative effects to biodiversity, natural environments, economics, and even human health. Exotic species become predators or competitors of native species (Hill and Lodge 1999), spread parasites or pathogens (Torchin et al. 2003, Torchin and Mitchell 2004), cause unexpected hybridization (Gaskin and Schaal 2002, Mallet 2007), and may ultimately cause a decrease in local biodiversity by eliminating local species (Chapin et al. 2000, Dick and Platvoet 2000). The sailfin catfish, a collection comprising several morphologically similar species

from the suckermouth armored catfish family Loricariidae, are among one of the exotic fish groups most seriously threatening tropical and subtropical freshwater regions (Liang et al. 2005, Chavez et al. 2006, Nico 2010). Widely believed to be an excellent algae grazer, these fish have been introduced all around the world from South America by the aquarium trade. Nevertheless, occasional escapes due to carelessness of owners provided a chance for these fish to establish populations in tropical and subtropical freshwater bodies. Ecological impacts of this fish include

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competition with native algae-grazers, direct predation on bottom-attached eggs of other fish, bank destabilization, increased turbidity, and economic loss to fisheries (Chavez et al. 2006, Nico 2010). Fishermen blame them for decreasing marketable fish in rivers, and destroying fishing equipment (Chavez et al. 2006; also encountered in our fieldwork in Taiwan).

The invasion of sailfin catfish in Taiwan can be traced to the late 1970s, when this kind of fish first appeared in the local ornamental fish trade (Liang et al. 2005). In the beginning, some of the fish were directly imported from South America, presumably Brazil or Peru. Some others may have been introduced from farm-raised individuals, possibly from Florida, USA. In the early 1980s, captive breeding was successfully established in Taiwan, and the offspring of such populations have become available to meet the great demand of local pet shops (Jun-Yi Gu, and Shen-Horn Yen, pers. comm.). Although the 1st formal record was not published until 1996 (Jiang 1996), we believe that the appearance of this fish in the wild in Taiwan can possibly be traced earlier to the late 1980s or early 1990s. With a large brood size, parental care behavior, air-breathing ability, excellent tolerability of highly polluted water, and lack of predators of adults in invaded areas (Armbruster 1998, Liang et al. 2005), this fish soon spread around western Taiwan and became one of the most dominant fish species in ponds, lakes, and downstream sections of rivers (Liang et al. 2005). Recently, this fish was listed as one of the top 30 invasive species in Taiwan (Lee et al. 2006).

Although it has such serious potential impacts, the taxonomic status of the sailfin catfish in Taiwan has never been clearly identified. Different scientific names, including different genera and species, have been applied to describe the species occurring in Taiwan (references listed in Table 1). The earliest application of *Plecostomus punctatus* (Chang-Chien et al. 1996 2000, Cheng et al. 1997a b) is obviously an incorrect combination since *Plecostomus* has not been recognized as a valid generic name since the 1960s (fishbase: http://www.fishbase.org/home.htm). Other reports of *Hypostomus* sp. (Chang-Chien et al. 2005, Tsai et al. 2005) are also incorrect identifications, since this species group was removed from *Hypostomus* due to their more dorsal-fin rays (Weber 1991 1992). *Liposarcus* was later used by Lee and Gu (2004), Liang et al. (2005), Lee et al. (2006), and Lin (2007). This genus was once separated from *Pterygoplichthys* (Weber 1991 1992), but was later regarded as a synonym of *Pterygoplichthys* (Armbruster 2004). Thus, the exotic suckermouth armored catfish occurring in Taiwan should be *Pterygoplichthys* species. However, to which species do they belong?

The use of specific names for this catfish is also problematic. Han and Fang (1997) first used *P. gibbiceps* without a further detailed interpretation. As the 2nd-most common Loricariid in the local aquarium trade, the occasional release of this species into the rivers cannot be excluded. Another possible candidate is *P. joselimaianus*, which is morphologically similar to *P. gibbiceps*, and is also very common in aquarium stores. Nevertheless, with much lower tolerance to highly polluted waters and low water temperatures in winter (pers. obs. based on our long-term experiments in keeping Loricariids), we do not think these 2 species could survive for a long period in the wild. Furthermore, a live *P. gibbiceps* is easily distinguished by its large, circular, uniform markings on the entire body, along with its elongated fan-shaped dorsal and caudal fins; while *P. joselimaianus* is characterized

Table 1. Scientific names applied to sailfin catfish in Taiwan

Year	Names used	References
1996-2000	*Plecostomus punctatus*	Chang-Chien et al. 1996; Cheng et al. 1997a; Cheng et al. 1997b; Chang-Chien et al. 2000
1997	*Pterygoplichthys gibbiceps*	Han and Fang 1997
1999-2003	*Pterygoplichthys* sp.	Chen and Fang 1999; Chang-Chien et al. 2002, Shao and Chen 2003
2003-2006	*Pterygoplichthys multiradiatus*	Chen et al. 2003; Lin et al. 2004; Lee et al. 2006
2004-2007	*Liposarcus pardalis*	Lee and Gu 2004; Lin 2007
2005	*Hypostomus plecostomus*	Chang-Chien et al. 2005; Tsai et al. 2005
2005	*Liposarcus multiradiatus*	Liang et al. 2005
2006	*Pterygoplichthys disjunctivus*	Page and Robins 2006

by short vermiculations on the sides of its body. A preliminary check of the fish we collected in rivers excluded the existence of these 2 species in the wild.

Pterygoplichthys multiradiatus, *P. pardalis*, and *P. disjunctivus* were later applied by different authors. Because of their similar morphologies, distinguishing among these 3 species caused the main disagreement. *Pterygoplichthys multiradiatus* has widely established populations around the world especially in North America (see references in Table 2). This name is most commonly applied by local authors (Chen et al. 2003, Lin et al. 2004, Liang et al. 2005) but without further explanations. In the current situation, this species is officially recognized in government publications (Lee et al. 2006). However, Lee and Gu (2004) and Lin (2007) both proposed that the exotic fish was *Liposarcus pardalis* (= *Pterygoplichthys pardalis*) based on their experience with aquarium species. Page and Robins (2006) proposed the existence of *P. disjunctivus* in Taiwan, but the number of evaluated specimen was limited (only 1 individual from the Kao-Ping River). With this ongoing debate, determining which species exist in Taiwan remains problematic. This situation highlights the urgent necessity for a precise identification of the sailfin catfish in Taiwan.

On the other hand, investigations from neighboring countries indicate the possibility of the coexistence of more than 1 species (Table 2). In Singapore, Indonesia, and the Philippines, both *P. pardalis* and *P. disjunctivus* were recorded in the wild with sympatric distributions (Kottelat and Whitten 1993, Chavez et al. 2006, Page and Robins 2006). Distinguishing between the 2 species is mainly based on their ventral spot patterns: *P. disjunctivus* is covered with vermiculations, while *P. pardalis* is covered with spots (Armbruster and Page 2006, Page and Robins 2006). According to our preliminary observations, both types coexist in rivers of Taiwan, while the occurrence of intermediate forms is frequent (Fig. 1). We considered the possibility that the invasion of sailfin catfish was not restricted to a single species.

In this paper, samples were collected from 12 localities covering the major drainage basins in Taiwan where the fish have invaded. Morphological and genetic traits were carefully evaluated and compared between fish we caught in the wild and several common *Pterygoplichthys* species. Our 1st purpose was to precisely identify these common exotic species; the existence of *P. multiradiatus*, *P. gibbiceps*, and *P. joselimaianus* was rejected based on morphological and molecular comparisons. Second, we observed an increase in morphological variation and genetic diversity in invasive populations compared to a native *P. pardalis* population collected from the central Amazon River at Manaus, Brazil. We suggest the possibility that hybrid superiority between *P. pardalis* and *P. disjunctivus* might have increased the fitness of their offspring, and facilitated the invasion of this hybrid population into novel environments; thus, causing the

Table 2. Invasion records of the 3 most common sailfin catfishes (*Pterygoplichthys* spp.) in the world

Locality	*P. pardalis*	*P. disjunctivus*	*P. multiradiatus*	References
Asia				
Indonesia	✓	✓		Kottelat and Whitten 1993, Page and Robins 2006
Japan		✓		Japan Ministry of Environment 2005
Malaysia	✓			Page and Robins 2006, Samat et al. 2008
Philippine	✓	✓		Chavez et al. 2006
Singapore	✓	✓		Page and Robins 2006
Turkey		✓		Özdilek ŞY 2007
Bangladesh			✓	Hossain et al. 2008
Vietnam	✓			Levin et al. 2008
India			✓	Krishnakumar et al. 2009, Knight 2010
North America				
Puerto Rico			✓	Bunkley-Williams et al. 1994
Florida, USA		✓	✓	Shafland 1996, Gibbs et al 2008, Nico 2010
Mexico	✓		✓	Wakida-Kusunoki et al. 2007
Hawaii, USA			✓	Nico 2010

✓: occurrence of that species in literature.

serious ecological impacts that can be observed nowadays.

MATERIALS AND METHODS

Sample collection and identification

During 2008-2010, 145 adult sailfin catfish were collected from 12 localities in 10 Taiwanese drainage basins (Fig. 2). Specimens were identified based on keys provided by Weber (1991 1992) and Armbruster and Page (2006). The key characters distinguishing among the most similar 3 species were as follows: *P. multiradiatus* has discrete dark spots on the lateral and caudal peduncle, never coalescing or forming chevrons; *P. pardalis* has dark spots on the lateral and caudal peduncle coalescing or forming chevrons, with ventral spots mostly discrete; and *P. disjunctivus* has dark spots on the lateral and caudal peduncle coalescing or forming chevrons, while ventral spots coalesce to form vermiculations.

Because of the common occurrence of inter-mediate forms in our samples (Fig. 1), each specimen in this study was separately identified by all 3 authors. Only specimens with a congruent identification were given a certain scientific name. The remaining specimens, occupying roughly 30% of the samples, were assigned to an "intermediate form" category.

According to our investigation, almost all of the sailfin catfish occurring in fish shops were obtained from local tropical fish breeders, and transported throughout Taiwan. It is reasonable to doubt whether invasive populations were more likely to have been founded by captive-bred individuals instead of wild fish directly imported from their original habitat. Hence, we sampled 11 common sailfin catfish from 11 different fish shops throughout the island to represent a population of this fish in captivity. We also sampled 5 typical *P. gibbiceps* and 3 *P. joselimaianus* in order to detect possible invasion or genetic introgression by these 2 species in the wild. Finally, we obtained 10 sailfin catfish directly collected from Manaus, Brazil, which represented a native, pure-line *P. pardalis* from the central Amazon River. Since we found no individual matching the morphological traits of *P. multiradiatus* in the wild, we used a
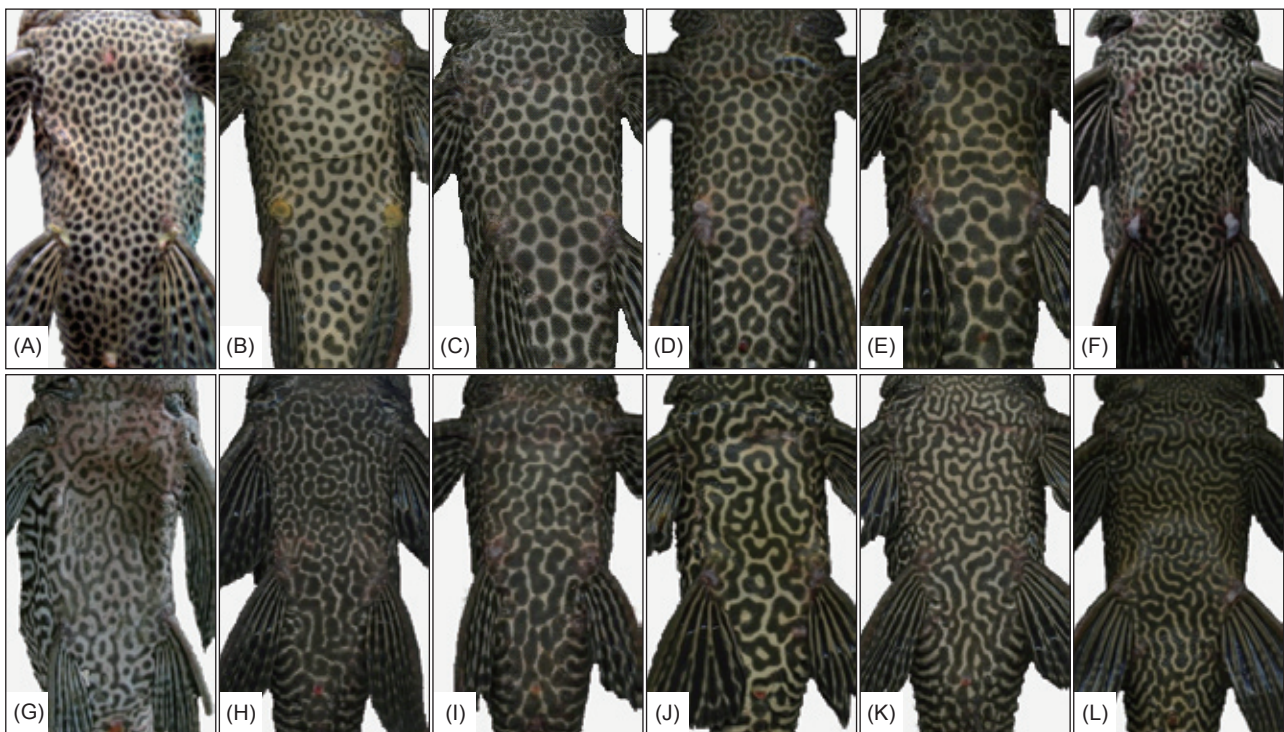

Fig. 1. Variations in ventral spots of the exotic sailfin catfish. Photo A, Native individual of *Pterygoplichthys pardalis* from the Amazon River at Manaus, Brazil, with discrete ventral spots; B and C, exotic individuals identified as *P. pardalis* in Taiwan; K and L, exotic individuals identified as *P. disjunctivus*, with ventral vermiculations; and D-J, a series of intermediate forms between the former 2 species from exotic populations.
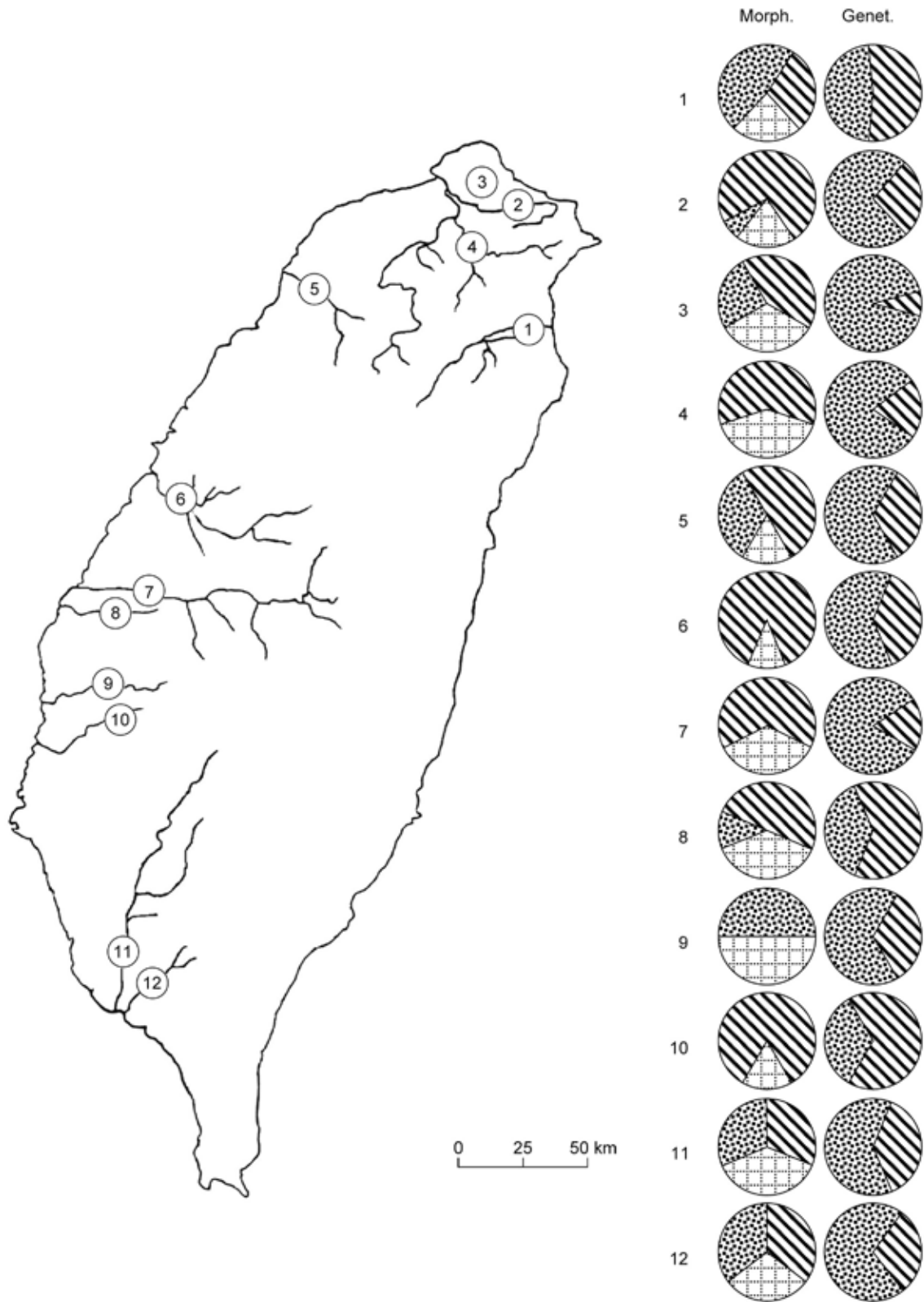
Fig. 2. Sample localities, morphology, and genetic composition of 12 exotic sailfin catfish populations in Taiwan. Pies on the left side indicate morphological proportions of *Pterygoplichthys pardalis*, *P. disjunctivus*, and the intermediate form respectively represented by dots, stripes, and cross squares. Pies on the right side indicate genetic proportions of the p clade and d-like clade, also respectively represented by dots and stripes.

DNA sequence from GenBank (accession no.: DQ119420) to represent the typical sequence of this species. *Hypostomus robinii* (DQ133770) and *Hemiancistrus maracaiboensis* (DQ119380) were included as outgroups (Hardman 2005).

DNA extraction, polymerase chain reaction (PCR), and sequencing reactions

Genomic DNA was extracted from muscle tissues using a LiCl method (Gemmell and Akiyama 1996). After ethanol precipitation, DNA was suspended in double-distilled H_2O and stored at -20°C. The complete sequence of the mitochondrial cytochrome (Cyt) *b* gene was amplified with primers L1 (5'-AAATACGGCGCA GGATTAGAAGCAAC-3') and H2 (5'-GGGAGT TAAAATCTCTCTTTTCTGGC-3'). PCRs were carried out in a 20-μl volume with the following thermal cycles: 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 30s, 55°C for 40 s, and 72°C for 90 s; with a final cycle at 72°C for 10 min. PCR products were run on 1.5% agarose gels in 1 × TBE buffer to ensure that the lengths of the target fragments were correctly amplified. DNA sequencing reactions were conducted on an ABI 3730 DNA Analyzer (Applied Biosystems, CA, USA) using the same primers used for the PCRs.

Obtained sequences were checked and assembled into contiguous arrays using Sequencher 4.8 (GeneCode, Boston, MA, USA). Primer regions were cropped, and the datasets were aligned using ClustalW in MEGA 4.0 (Tamura et al. 2007) with the default settings (a gap opening penalty of 10 and a gap extension penalty of 0.2). The entire procedure was repeated for individuals with rare haplotypes (there were 4 haplotypes with a sample size of < 2) to prevent errors from occurring during the PCR amplification. The aligned dataset contained a 1211-bp fragment, including 71-bp tRNA-Glu and 1140-bp Cyt *b* complete sequences. The obtained haplotypes were submitted to GenBank (accession nos.: HQ267774-HQ267783).

Data analyses and haplotype genealogy

General genetic information for each population, such as the number of haplotypes, haplotype diversity (h), and nucleotide diversity (π), were calculated using Dnasp vers. 5 (Librado and Rozas 2009). The genealogy among catfish haplotypes was represented by (1) a phylogenetic tree with other *Pterygoplichthys* spp. as outgroups and (2) a haplotype network without outgroups. Three phylogenetic methods, including maximum-likelihood (ML), maximum-parsimony (MP), and Bayesian inference (BI) analyses, were used to infer the phylogenetic relationships. ML was performed in PHYML (Guindon and Gascuel 2003) using the TrN + G model (Tamura-Nei model, Tamura and Nei 1993; with a gamma distribution value) suggested by the hierarchical likelihood ratio test in Modeltest 3.7 (Posada and Crandall 1998). A tree topology search was set to subtree pruning and regrafting (SPR). The initial tree used the default option (BIONJ), nucleotide frequencies were based on an "empirical" option, and the categories of the discrete gamma model were set to 4. Bootstrap analyses based on 2000 pseudoreplicates were carried out to evaluate the support of all branching relationships. MP was performed in PAUP* 4.0 b10 (Swofford 2002) with heuristic searches, starting trees determined by 1000 random taxon additions, the tree bisection-reconnection (TBR) branch swapping algorithm, gaps treated as missing data, and all characters treated as equally weighted. A strict consensus tree was computed in cases where multiple equally parsimonious trees were obtained. The topologies of the MP trees were evaluated by 2000 bootstrap replicates (Felsenstein 1985). The BI was carried out using MrBayes vers. 3.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Six chains (5 heated and 1 cold) were run for 10^6 generations, and trees were sampled every 100 generations. The 1st 2500 trees were treated as burn-in, and the remaining trees were used to represent the posterior probability. Finally, the haplotype network was constructed with the software TCS 1.21 based on the principle of parsimony (Clement et al. 2000). Each branch in the network was supported by a minimum probability threshold of 0.95 (Templeton et al. 1992).

RESULTS

Morphological identification of the sailfin catfish in the wild

Among the 145 exotic individuals evaluated, 31 (21.4%) were congruently identified by the 3 authors as *P. pardalis*, 73 (50.5%) presented external traits of *P. disjunctivus*, and 41 (28.3%) were assigned to the intermediate form (Fig. 2, Table 3). The fish collected from Manaus, Brazil

turned out to be *P. pardalis*. Surprisingly, we found no individual showing external traits identical or similar to *P. multiradiatus* (with discrete spots on the lateral sides and caudal peduncle), which was the most commonly applied species in the literature. Neither *P. gibbiceps* nor *P. joselimaianus* was found in the wild.

Seven of the 12 sample localities presented the coexistence of the 2 morphological forms (Fig. 2). *Pterygoplichthys pardalis* was not collected from the Xindian, Dadu, Zhuoshui, or Jiangjun Rivers (nos. 4, 6, 7, and 10); while *P. disjunctivus* was absent from the Jishui River (no. 9). Nevertheless, all 12 sample localities contained individuals of the intermediate form. They occupied more than 30% of individuals in 6 populations, and > 40% in 3 populations. The ratio of intermediate fish exceeded 10% in all populations (Fig. 2, Table 3).

Molecular data: basic information

Six haplotypes (P1, P2, P3, P4, D1, and D2) were obtained from the exotic sailfin catfish populations. Individuals from fish shops contained 4 haplotypes, including one that was not found in the wild (D3). All fish collected from Manaus, Brazil belonged to a single haplotype (P1), which was also the most common one among all individuals. Sequencing of the 5 *P. gibbiceps* yielded 2

haplotypes (G1 and G2), and 3 *P. joselimaianus* yielded 1 haplotype (J1).

Haplotype and nucleotide diversities of each sample locality are listed in table 3. The overall haplotype and nucleotide diversities of the exotic populations in Taiwan were 0.6263 and 0.0041, respectively. With 5 haplotypes, the Keelung River (no. 2) had the highest haplotype diversity (h = 0.7714) among all localities. Some other populations with only 2 haplotypes showed comparatively lower haplotype diversity. In contrast, the population collected near Manaus, Brazil showed extremely low genetic diversity due to the single haplotype.

Haplotype genealogy

The ML analysis generated a single tree with a log-likelihood (-lnL) value of 2826.38. The MP and BI showed similar results to the ML, with only 2 minor conflicts: (1) the within-species relationship among *P. pardalis* haplotypes differed; and (2) the monophyly of *P. gibbiceps* and *P. joselimaianus* was not supported by the BI analysis. Neither conflict affected our final conclusions.

The phylogenetic reconstruction showed that the 7 exotic haplotypes could be further divided into 2 clades. Since the pure *P. pardalis* from the natural habitat represented a single haplotype (P1), we defined the clade containing this haplotype as

Table 3. Sample size (*n*), number of haplotypes (N_{hap}), haplotype diversity (H), nucleotide diversity (π), morphology, and genetic proportions of each exotic sailfin catfish (*Pterygoplichthys*) population used in this study

Population		*n*	N_{hap}	H	π	Morphological statistics	Haplotype statistics
						p form/ d form/ int. form	p clade / d-like clade
Exotic population in Taiwan		145	6	0.6263	0.0041	31 / 73 / 41	94 / 51
1	Lanyang Stream	17	2	0.5294	0.0044	8 / 5 / 4	8 / 9
2	Keelung River	15	5	0.7714	0.0040	1 / 11 / 3	11 / 4
3	Dahu Park	12	4	0.6364	0.0021	3 / 5 / 4	11 / 1
4	Xindian Stream	5	2	0.4000	0.0033	0 / 3 / 2	4 / 1
5	Keya Stream	12	2	0.4849	0.0040	4 / 6 / 2	8 / 4
6	Dadu Stream	8	3	0.7500	0.0048	0 / 7 / 1	5 / 3
7	Zhuoshui Stream	17	2	0.3088	0.0026	0 / 11 / 6	14 / 3
8	Beigang Stream	8	2	0.5357	0.0044	1 / 4 / 3	3 / 5
9	Jishui Stream	6	3	0.7333	0.0047	3 / 0 / 3	4 / 2
10	Jiangjun Stream	12	3	0.5303	0.0041	0 / 10 / 2	4 / 8
11	Kaoping Stream	16	2	0.5000	0.0042	5 / 5 / 6	10 / 6
12	Donggang Stream	17	4	0.7279	0.0042	6 / 6 / 5	12 / 5
Aquarium fish in Taiwan		11	4	0.7455	0.0046	N/A (juveniles)	8 / 3
P. pardalis from Manaus, Brazil		10	1	0.0000	0.0000	11 / 0 / 0	10 / 0

p form: pardalis form; d form: *disjunctivus* form; int. form: intermediate form; p clade: *pardalis* clade; d-like clade: *disjunctivus*-like clade.

the *pardalis* clade (p clade), and the other as the *disjunctivus*-like clade (d-like clade) (Fig. 3). All individuals collected from the wild belonged to these 2 clades with no exceptions (the shaded area in Fig. 3). This result is congruent with the morphological observations, that *P. multiradiatus*, *P. gibbiceps*, and *P. joselimaianus* were all absent from the wild. Among the 145 exotic individuals, the p clade contained 4 haplotypes and 94 indivi-

duals; while the d-like clade contained 2 haplotypes and 51 individuals. Both clades coexisted in all 12 exotic populations (Fig. 2), with the fish shop population showing a similar phenomenon. The 7 exotic haplotypes were further used to construct a haplotype network (Fig. 4). The 2 clades were separated by a 10-step connection.
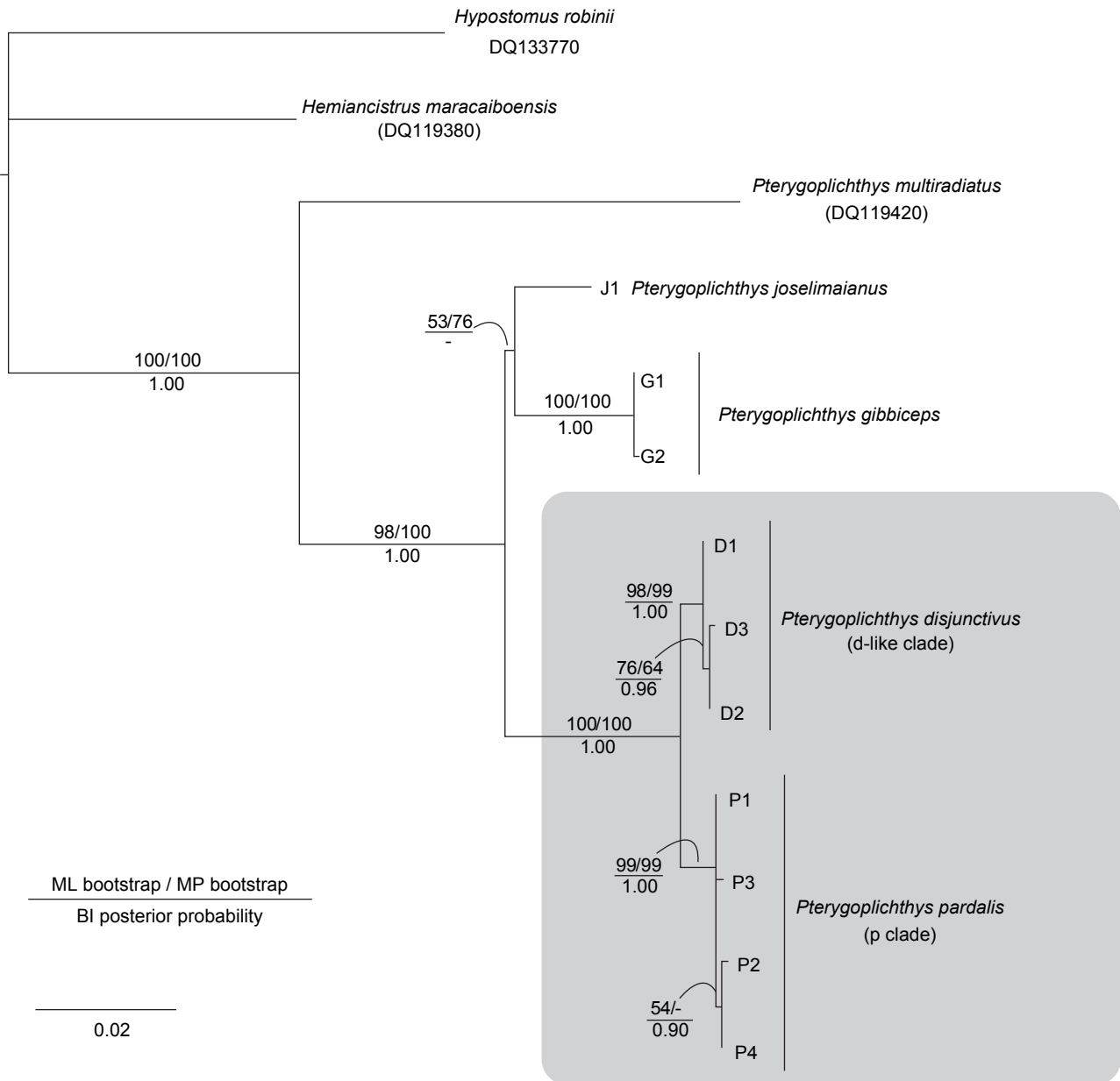

Fig. 3. Maximum-likelihood (ML) tree of sailfin catfish haplotypes based on mitochondrial cytochrome *b* sequences. Statistical support at each node was evaluated by ML bootstraps, maximum-parsimony bootstraps, and Bayesian posterior probabilities. All individuals collected from the wild are limited to the shaded area, thus excluding the occurrence of *Pterygoplichthys multiradiatus*, *P. joselimaianus*, and *P. gibbiceps* in rivers of Taiwan.

Inconsistency between morphological and genetic traits

Morphological traits in the exotic populations did not fit the results of the molecular markers. Only 21 among the 31 fish exhibiting the *P. pardalis* morphology possessed a p clade sequence, while only 28 of the 73 fish (38%) exhibiting the *P. disjunctivus* morphology had a d-like clade sequence (Table 4). Both p clade and d-like clade haplotypes contained fish representing *pardalis*, *disjunctivus*, and the intermediate forms. On the other hand, some fish with very different color patterns showed identical sequences. The most dominant haplotypes were P1 and D1, and they occurred in all exotic populations containing a variety of different color morphs (Fig. 4). The entire trend showed that 64% of the fish carried p clade sequences, and 36% of the fish carried d-like sequences. These results show that the morphological and genetic traits have been completely homogenized with no differentiation.

DISCUSSION

Based on our morphological identification and molecular data, exotic sailfin catfish in Taiwan should be identified as *P. pardalis*, *P. disjunctivus*, or a mixture between the two. *Pterygoplichthys multiradiatus*, another exotic species commonly recorded in other countries and currently listed in official records, was not found in Taiwanese rivers. Considering records in neighboring regions (Table 2), it seems that invasion of this species has not yet occurred in East Asia. Furthermore, we also excluded the possibility of population invasion or genetic introgression from *P. gibbiceps* and *P. joselimaianus*, both of which are very common species in local fish shops and the aquarium trade. According to our long-term experiments in keeping loricariids, these 2 species have lower tolerance to highly polluted water and low water temperatures, which prevent the successful establishment of stable populations.

However, the apparent mismatch between the color patterns and DNA lineages of the sailfin catfish raises several possibilities. Here, we put forth 2 hypotheses and a speculation to explain the current situation. First, *P. disjunctivus* might just be a synonym of *P. pardalis*. Second, the exotic population may have originated from hybridization between the 2 species or allopatric populations. Third, we suspect that the superiority of the hybrid might have helped increase its fitness during population invasions.

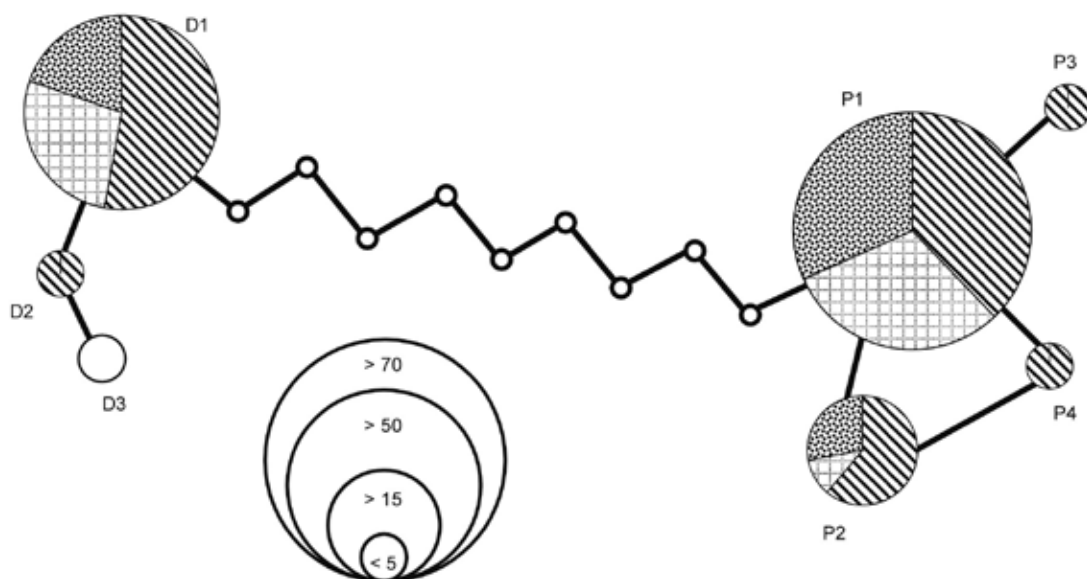## The validity of *P. disjunctivus* remains an open question

Fig. 4. Haplotype network of the exotic sailfin catfish. The size of each circle of a haplotype indicates the sample size. The pie charts represent the morphological proportions of *Pterygoplichthys pardalis*, *P. disjunctivus*, and the intermediate form which are respectively represented by dots, stripes, and cross squares.

Our results indicated totally fluent gene flow between individuals with ventral spots and those with ventral vermiculations. Morphological and genetic traits between the 2 groups were completely homogenized with no differentiation and a large proportion of individuals representing intermediate forms. Under this situation, *P. disjunctivus*, the latter published species, is possibly just a synonym of *P. pardalis*. The 2 clades within the entire population represent a 10-bp divergence, yielding a 0.93% genetic distance. Compared to other interspecific divergences among *Pterygoplichthys* spp., this value is too low to support the 2 clades being 2 distinct species. To answer this question, specimens from the type locality representing pureline *P. disjunctivus* are needed. Nevertheless, according to the current data, prematurely invalidating this species based on insufficient evidence would cause taxonomic controversy. We suggest a taxonomic revision of this species group if more specimens from the original habitats are available in the future. Before that, we tentatively leave the validity of *P. disjunctivus* an open question.

Multiple invasions and hybridization

The status of the validity of *P. disjunctivus* does not change our 2nd prediction that the current exotic populations in Taiwan originated from a mixture between 2 species or multiple populations. This prediction is based on the observation that the population from the central Amazon River Manaus, Brazil, presented neither molecular nor morphological variations. Haplotypes belonging to the d-like clade did not appear in that population, while ventral vermiculations were absent as well. If this represents the condition of the pure-line *P. pardalis*, we suspect that the character of ventral

vermiculations was introduced from 1 or more other populations. According to the current taxonomy, it should be defined as hybridization between *P. pardalis* and *P. disjunctivus*. Even if *P. disjunctivus* becomes a synonym, the exotic populations were still deduced to have originated from outbreeding between 2 allopatric lineages. The original habitat of *P. pardalis* is from the major drainage of the Amazon River, while typical *P. disjunctivus* is from a southern drainage basin, the Madeira River (Weber 1992). According to their native distributions, it is reasonable to suspect that the aquarium trade was mainly responsible for having brought these populations together. The existence of 2 clades indicates that they were introduced to Taiwan from multiple sources. Therefore, the high genetic variability of Taiwanese populations might have been due to multiple invasions.

Speculation: Is the superiority of the hybrids the cause?

Typically, exotic populations are characterized by lower genetic diversity due to founder effects or population bottlenecks (Sakai et al. 2001, Allendorf and Lundquist 2003). Reduced genetic variation limits the adaptability of such populations to novel environments, and increases the risk of extinction (Frank and Ralls 1998, Frankham et al. 2002, Allendorf and Lundquist 2003, Dlugosch and Parker 2008). Nevertheless, exotic populations can gain higher genetic variation if multiple invasions or hybridization occurs (Grosholz 2002). A case in plants indicated that hybridization between *Tamarix ramosissima* and *T. chinensis* induced a serious invasion of areas in the western US, causing serious ecological problems (Gaskin and Schaal 2002). Another case occurred when a non-native genotype invaded native reeds (*Phragmites australis*), and caused a dramatic increase of the population in the last 150 yr (Saltonstall 2002). Compared to plants, evidence from animals is restricted. Studies in the lizard, the brown anole (*Anolis sagrei*), indicates that a genetic admixture from different native populations may have caused an increase in fitness; thus, providing a chance for their distributional spread (Kolbe et al. 2004 2007 2008).

Referring to these cases, we propose the possibility that hybridization between *P. pardalis* and *P. disjunctivus* might have similarly increased the fitness of the exotic sailfin catfish. They currently successfully occupy downstream reaches of most major rivers of western Taiwan. In many

Table 4. Comparison between morphological identification and genetic clades for all sailfin catfish (*Pterygoplichthys*) collected in the wild in Taiwan

	p clade	d-like clade	Total
P. pardalis	21	10	31
P. disjunctivus	45	28	73
Intermediate form	27	14	41
Total	93	52	145

p clade: *pardalis* clade; d-like clade: *disjunctivus*-like clade.

regions, water bodies are so seriously polluted that no other fish can survive except for these catfish. We expect this to be another case representing the serious impacts of invasions gaining novel fitness when their genetic diversity increases by human-induced outbreeding or ex situ hybridization. Obtaining pure-line *P. disjunctivus* specimens and analyzing them using nuclear markers in future studies will provide critical evidence for this. Furthermore, comparative experiments on the tolerance of these sailfin catfish might be important as well.

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