

*Genetic structure in the Amazonian catfish
Brachyplatystoma rousseauxii: influence of
life history strategies*

**F. M. Carvajal-Vallejos, F. Duponchelle,
E. Desmarais, F. Cerqueira, S. Querouil,
J. Nuñez, C. García & J.-F. Renno**

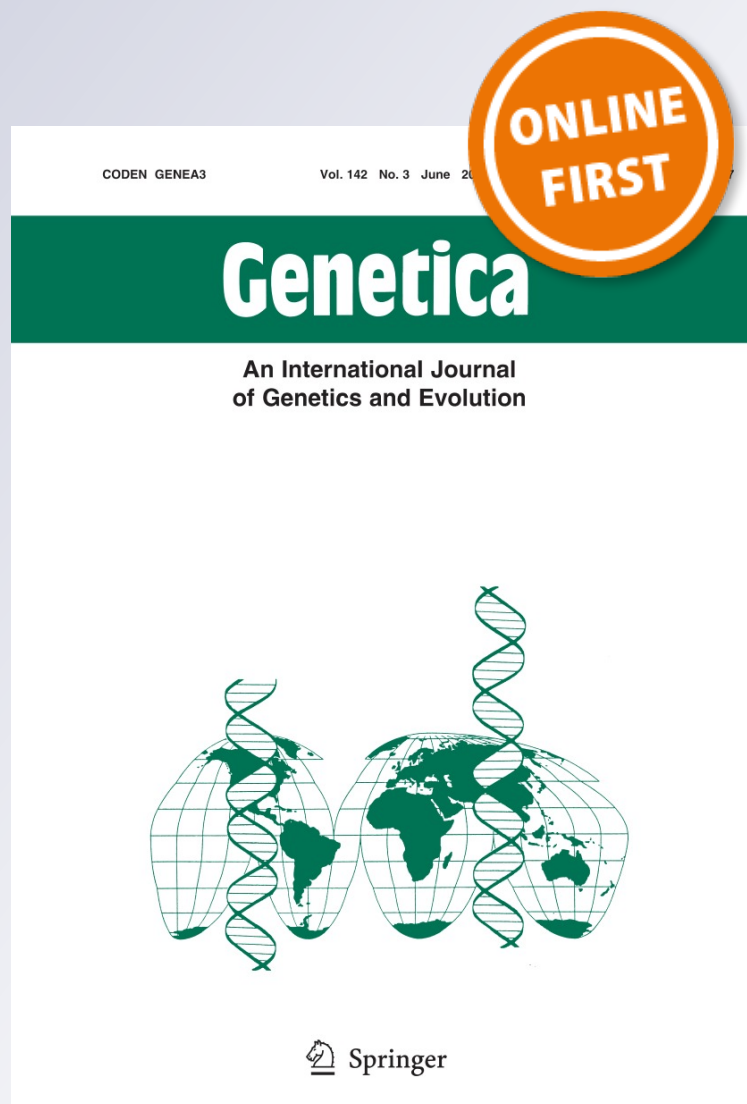
Genetica

An International Journal of Genetics and
Evolution

ISSN 0016-6707

Genetica

DOI 10.1007/s10709-014-9777-2



Your article is protected by copyright and all rights are held exclusively by Springer International Publishing Switzerland. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Genetic structure in the Amazonian catfish *Brachyplatystoma rousseauxii*: influence of life history strategies

F. M. Carvajal-Vallejos · F. Duponchelle ·
E. Desmarais · F. Cerqueira · S. Querouil ·
J. Nuñez · C. García · J.-F. Renno

Received: 21 December 2013 / Accepted: 7 July 2014
© Springer International Publishing Switzerland 2014

Abstract The Dorado or Plateado (Gilded catfish) *Brachyplatystoma rousseauxii* (Pimelodidae, Siluriformes) is a commercially valuable migratory catfish performing the largest migration in freshwaters: from the Amazonian headwaters in the Andean foothills (breeding area) to the Amazon estuary (nursery area). In spite of its importance to inform management and conservation efforts, the genetic variability of this species has only recently begun to be studied. The aim of the present work was to determine the population genetic structure of *B. rousseauxii* in two regions: the Upper Madera Basin (five locations in the Bolivian Amazon) and the Western Amazon Basin (one regional sample from the Uyucaí–Napo–Marañón–Amazon basin, Peru). Length polymorphism at nine microsatellite loci (284 individuals) was used to determine genetic variability and to identify the most probable panmictic units (using a Bayesian approach), after a significant departure from Hardy–Weinberg equilibrium was observed in the overall dataset (Western Amazon + Upper Madera).

Bayesian analyses revealed at least three clusters in admixture in the five locations sampled in the Bolivian Amazon, whereas only two of these clusters were observed in the Western Amazon. Considering the migratory behaviour of *B. rousseauxii*, different life history strategies, including homing, are proposed to explain the cluster distribution. Our results are discussed in the light of the numerous threats to the species survival in the Madera basin, in particular dam and reservoir construction.

Keywords Bolivia · Madera River · Population structure · Microsatellites · Siluriformes · Pimelodidae

Introduction

Brachyplatystoma rousseauxii (Castelnau 1855) is a large (>1.5 m) migratory catfish of the family Pimelodidae, commonly known as Dorado in Bolivia, Colombia and

F. M. Carvajal-Vallejos · F. Duponchelle · E. Desmarais ·
S. Querouil · J. Nuñez · C. García · J.-F. Renno
Laboratoire Mixte International – Evolution et Domestication de
l'Ichthyofaune Amazonienne (LMI – EDIA), Centro de
Investigaciones Quistococha, Iquitos-Loreto, Perú

F. M. Carvajal-Vallejos
FAUNAGUA (Institute for Applied Research on Aquatic
Resources), final Av. Max Fernández s/n, zona Arocagua Norte,
Municipio de Sacaba, Cochabamba, Plurinational State of
Bolivia

F. M. Carvajal-Vallejos (✉)
Unidad de Limnología y Recursos Acuáticos (ULRA), Facultad
de Ciencias y Tecnología (FCyT), Universidad Mayor de San
Simón (UMSS), calle Sucre frente al Parque La Torre s/n, zona
Las Cuadras, Cochabamba, Plurinational State of Bolivia
e-mail: fmcvalle@yahoo.com

F. Duponchelle · S. Querouil · J. Nuñez · J.-F. Renno
Institut de Recherche pour le Développement (IRD), UMR-
BOREA, 911 Avenue Agropolis, 34394 Montpellier, France

E. Desmarais · F. Cerqueira
Centre National de la Recherche Scientifique (CNRS), UMR-
ISEM (Institut des Sciences de l'Evolution de Montpellier),
Université Montpellier 2, Bâtiment 22, C.C. 065,
34095 Montpellier Cedex 5, France

C. García
Instituto de Investigaciones de la Amazonía peruana (IIAP),
Carretera Iquitos-Nauta, km 4.5, Quistococha, Iquitos, Perú

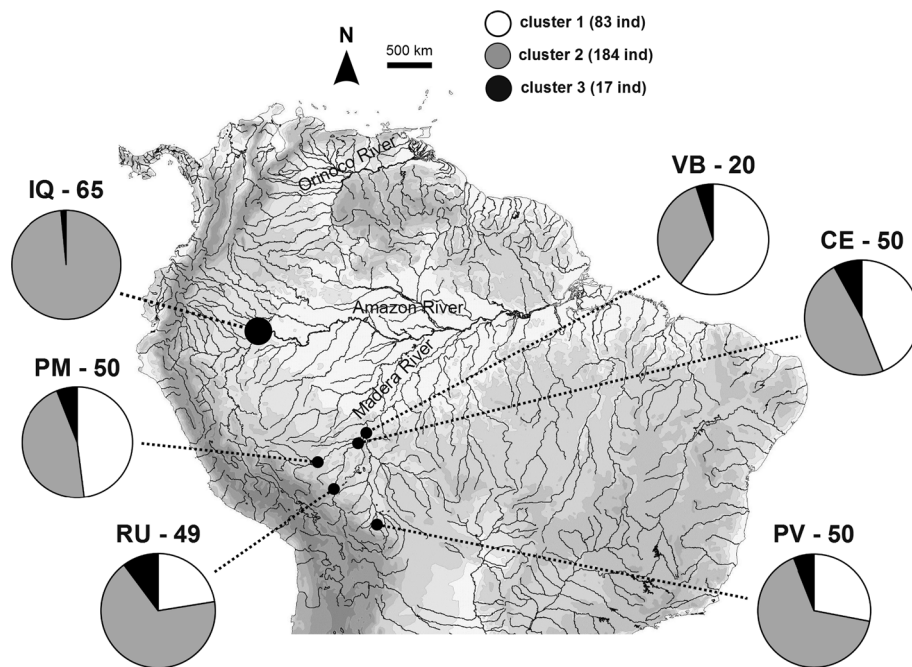
Peru, as Dourada in Brazil and as Plateado in Bolivia (Carvajal-Vallejos et al. 2011). It is one of the most emblematic species of the Amazon basin, owing to its economic importance and exceptional life cycle, involving the largest known migration in freshwater (Barthem and Goulding 1997, 2007; Alonso 2002; Araujo-Lima and Ruffino 2003). The Dorado is widely distributed throughout the white-water tributaries and main channels of the Amazon River (Araujo-Lima and Ruffino 2003; Barthem and Goulding 2007), and also can be observed sporadically in clear- and black-water rivers (e.g. Negro, Tocantins—Goulding 1980). There are few studies on this species to date, despite the interest that arose among ichthyologists as a result of Barthem and Goulding's (1997) hypothesis about its life cycle. These authors followed the size distribution of the Dorado among the main fishing ports along the Amazon and Lower Madera River channels, from the estuary (Belem) all the way to the headwaters of an Amazon tributary in Peru (Pucallpa, 4,500 km from the estuary). Fishery statistics and experimental fishing data collected over several years showed a clear change in size distribution of the Dorado between the estuary and the headwaters, with only small and medium-sized fish in the estuary, almost exclusively large specimens in the headwaters of Peru or Colombia, and intermediate size-classes in between. They also observed that juvenile Dorado were absent and pre-adults and adults were rarely found in the flood plains. Alonso (2002), using age determination from otolith readings, reconstructed the age-size distribution of the species along the main axis of the Amazon River. His results confirmed that while mostly juveniles were present in the Amazon estuary, almost exclusively adults were observed in the Iquitos area and mostly pre-adults were caught in between. Providing new information regarding age, growth, reproductive biology, and species' dependence on fluvial dynamics along the Amazon main stem, studies of García et al. (2009a) and Agudelo et al. (2013) confirmed the existence of a complex life cycle involving long-distance migration between the Amazon estuary and the headwaters. In the Peruvian and Colombian Amazonia, where these studies were carried out, *B. rousseauxii* reaches sexual maturity at about 4 years old and 90 cm SL and has longevity of at least 15 years (García et al. 2009; Agudelo et al. 2013).

Batista and Alves-Gomez (2006) suggested that the Dorado displays spatial genetic variability along the Amazon's mainstem that could be explained by a homing behaviour. These authors observed that haplotypic diversity decreases from the estuary westwards and suggested that this could be due to preferential recruitment of differentiated populations to certain tributaries as the fish move upstream. While an interesting hypothesis, the variability observed in this study also could have alternative

explanations since the sampling design and molecular markers used (the maternally-inherited Control Region—CR at mtDNA) did not provide sufficient information or consistent evidence of a philopatric migration model (see Campos Telles et al. 2011) and were unable to define the reproductive units related to a possible homing behaviour. More recently, Batista (2010), using nuclear (microsatellites) and mitochondrial (CR) DNA markers, concluded without testing departure from Hardy–Weinberg equilibrium (HWE) that the large number of individuals collected from different tributaries of the Amazon basin (including the Madera River) were parts of a single panmictic unit. The possibility of a homing phenomenon, however, was not totally dismissed, as some degree of structure was observed for mtDNA among some large tributaries. These conclusions, suggesting the existence of a single population of Dorado in the Amazon basin, could have dramatic consequences for species conservation. Indeed, the Dorado is considered overexploited in the Lower Amazon (Petreire et al. 2004; Alonso and Pirker 2005) and heavily exploited in some areas of the Upper Amazon in Peru (García et al. 2009a, b) and Colombia (Agudelo et al. 2013). If this species is composed of a large, single panmictic population of homogeneous individuals throughout its distribution area, then over-exploiting the species in some tributaries would not be particularly problematic at regional scale, as recruitment would be ensured from other, less exploited tributaries. On the other hand, if there is genetic structure within the species, then the total allowable catches have to be set and enforced for each population. Additionally, the growing number of hydroelectric power plants under construction in the Amazon basin are further threatening the species by disturbing or interrupting its migration routes (Junk et al. 2007; Van Damme et al. 2011b; Finer and Jenkins 2012). But again, if the single panmictic population hypothesis is verified, a depletion of the breeding individuals in the Upper Madera following the Madera hydroelectric impoundments of Santo Antônio and Jirau, might be regarded by dam supporters as not so dramatic (even though reducing the range of a species could be problematic), as recruitment for the population would be ensured from the Upper Amazon–Solimões system. Considering that the Dorado is an economically (e.g. Goulding 1979; Agudelo et al. 2000) and ecologically (Angelini et al. 2006) important, yet relatively poorly studied resource with a complex life cycle occurring over a continental scale while subject to differential fishing pressures (on juveniles in the Central Amazon Basin and on adults in the Western Amazon Basin), it is crucial to better understand its biology and genetic structure and shed new light on a potential homing behaviour.

The Amazon Basin is over 6,000,000 km², and its main tributaries (e.g. Madera, Ucuyalí) have distinct hydrological

Fig. 1 Composition and distribution of the clusters identified by BAPS for each geographic location studied in the Upper Madera (VB, CE, PM, RU, PV) and Western Amazon (IQ) (Bolivian and Peruvian Amazon). Numbers next to the location names refer to the number of individuals of *Dorado Brachyplatystoma rousseauxii* examined from each location. Locations correspond to VB: Villa Bella; CE: Cachuela Esperanza; PM: Puerto Maldonado; RU: Rurrenabaque; PV: Puerto Villaruel; IQ: Iquitos—larger black circle means a regional sampling. Base map of drainages and elevations provided by Conservation Science Program, World Wildlife Fund US



and geological characteristics. The Madera is one of the largest sub-basins of the Amazon, and among those that contribute the most in terms of water and sediment volumes (Barthem and Goulding 2007; Wilkinson et al. 2010; Molina and Vauchel 2011). Most of the upper portion of the Madera Basin is in Bolivian territory, fed by several tributaries with distinct physicochemical characteristics (Navarro and Maldonado 2002). These tributaries converge into one channel (the Madera River proper) that is separated from the Lower Madera portion by a series of rapids spread over 300 km (Molina 2011a). The history of this basin and the rapids (principally the Teotônio rapids in Porto Velho) have had an important influence as a dividing boundary on the structure of the aquatic organism and fish communities (Farias et al. 2010; Torrente-Vilara et al. 2011; Carvajal-Vallejos and Zeballos 2011; Tavera et al. 2011), and likely also on specific populations of species of migratory fishes able to pass the rapids (Goulding 1980; Araujo-Lima and Goulding 1997; Barthem and Goulding 1997), such as the Dorado. While little, apart from some preliminary information (Coronel et al. 2004), is known about the biology of *B. rousseauxii* in the Madera Basin, it could be expected that its migratory dynamics would be similar here to those described for the Amazon main channel. Given the size and particular characteristics of the Upper Madera, the species might present some degree of genetic structure, associated with specific geographic zones or sub-basins, with fish returning to and breeding in the area where they were born (i.e. homing).

This study aimed at generating new genetic information on geographic populations of Dorado present in the upper

basin of the Madera River (Bolivian and Peruvian Amazon), and to compare this with information obtained from fish in the Peruvian Amazon system. Sampling design and micro-satellite loci analysis were organized and selected specifically to assess genetic populations at different geographical scales (local and regional), and to test whether homing takes place towards the tributaries of the Upper Madera Basin.

Methods

Study area

The study area included the upper basin of the Madera River in Bolivia and Peru (Puerto Maldonado) (locations VB, CE, PM, RU, PV—Fig. 1), and the Ucayalí–Marañón–Napo–Amazon system around Iquitos (Peru) (location IQ—Fig. 1), defined hereafter as the Western Amazon. The Western Amazon system accounts for at least 22 % of the total water volume and represents almost 10 % of the total Amazon watershed area (Goulding et al. 2003). Its lower reaches have characteristics similar to those seen in the Central Amazon, and there is no apparent barrier separating them.

Sampling design

Considering existing information on the biology of *B. rousseauxii* and the variability of aquatic systems in the Upper Madera Basin (mainly the Bolivian Amazon), the sample collection strategy encompassed two geographic

scales for analysis: a local-scale (main tributaries of the Upper Madera River), and a regional-scale (main Amazon tributaries—Madera and Western Amazon systems).

Sample locations for the Upper Madera were defined according to the hydrological network and information gathered through personal interviews with fishermen between 2005 and 2006. Additionally, reference information on the biology and distribution of large pimelodids in the Madera Basin (e.g. Goulding 1979, 1980; Barthem et al. 1991, 2003; Barthem and Goulding 1997, 2007; Lauzanne et al. 1999) also was considered.

Each major, turbid-water tributary was sampled in its upper area. In order to assess the possibility that the species swims up-river in a selective manner and that major tributaries may contain distinct panmictic units, we also sampled in two spots close to the confluence of the major tributaries, Cachuela Esperanza and Villa Bella (Fig. 1).

All the samples from the Upper Madera were obtained from local fishermen directly on the fishing ground (which often required several weeks of travel with fishermen), and catch location was recorded. For the broader regional-scale analysis, samples were obtained from the market in Belén (Iquitos, Peru), where fisher families sold fish caught in an area of ~300 km around Iquitos, which includes the Amazon (Upper Solimões), Ucayalí, Napo and Marañon river systems. In total, 284 individuals of *B. rousseauxii* were collected between January 2005 and March 2009. Two hundred and nineteen Dorados were captured using gillnets (20–24 cm mesh size) in the main channels of the Madera (20 in Villa Bella—VB with a mean standard length—SL of 54 ± 14.5 cm estimated from only seven individuals), Upper Beni (49 in Rurrenabaque—RU), Lower Beni (50 in Cachuela Esperanza—CE, 91.8 ± 10.1 cm SL), Madre de Dios (50 in Puerto Maldonado—PM), and Ichilo or Upper Mamoré (50 in Puerto Villarroel—PV, 89.2 ± 8.2 cm SL) rivers. As size at first sexual maturity is ~75 cm in the Madera (M. Hauser UNIR—Brazil and F. Duponchelle, unpublished data), hence smaller than in the Peruvian and Colombian Amazonia (García et al. 2009a; Agudelo et al. 2013), most sampled individuals could be considered as adults, except the seven small specimens caught in VB. Sixty-five samples were purchased from the central fish market in Belén (Iquitos—IQ). For each individual caught or purchased, approximately 1 cm³ of muscle was preserved in 98 % ethanol.

For the Upper Madera and the Western Amazon, each locality is represented by samples from different periods of the year and different years.

DNA extraction

Genomic DNA was extracted from ethanol-preserved muscle following modifications of the CTAB procedure

developed by Doyle and Doyle (1987): proteinase K (10 µL/100 mg sample, 10 mg/mL) was added for DNA extraction for 10 h (60 °C), chloroform (1 mL) was used instead of chloroform-isoamyl alcohol to extract proteins and cell debris (8,000 rpm by 5 min), and a final 15 min of washing (13,000 rpm) with 70 % ethanol (750 µL), and resuspension of the nucleic acid pellet in water.

Microsatellite amplification

Microsatellite amplification was performed at 9 loci using fluorescently labeled (FAM, HEX, NED) primers. The primers used were designed for *Pseudoplatystoma corruscans* (Pcor1, Pcor2, Pcor 7) by Revaldaves et al. (2005) and *B. rousseauxii* (BR37, BR38, BR44, BR45, BR47, BR49) by Batista et al. (2009).

Microsatellite amplification by polymerase chain reaction (PCR) was carried out on a Mastercycler thermocycler (Eppendorf AG 2231, Hamburg). Fragments amplified with Pcor1, Pcor2 and Pcor7 primers were produced in a total volume of 10 µL consisting of 2.0 µL of 5× reaction buffer (Colorless GoTaq Promega, 7.5 mM MgCl₂ at pH 8.5), 0.6 µL of MgCl₂ (25 mM), 1.0 µL of dNTPs (1 mM each dNTP), 0.4 µL of each primer (forward and backward, 10 µM), 0.4 U of Taq polymerase (GoTaq, Promega), approximately 50 ng DNA extract and 4.02 µL Milli-Q water. The temperature profile to which the mixture was subjected consisted of an initial denaturation at 94 °C (2 min); followed by 35 denaturation cycles at 94 °C (30 s), annealing at 57 °C (Pcor1), 58 °C (Pcor2), 62 °C (Pcor7) (30 s), elongation at 72 °C (40 s); and one final elongation at 72 °C (30 min), followed by final refrigeration of the products at 4 °C.

Amplifications with the remaining primers (BR) were designed and executed in duplex 1 (BR37-BR38), duplex 2 (BR44-BR45), and duplex 3 (BR47-BR49). Compatibility and grouping of the primers for the formation of the duplexes were evaluated with the program MULTIPLEX version 1.2 (Kaplinski et al. 2004). All duplex reactions were carried out in a final volume of 10 µL, composed of 2 µL of 5× reaction buffer (Colorless GoTaq, Promega, 7.5 mM MgCl₂ at pH 8.5), 0.6 µL of MgCl₂ (25 mM), 2.0 µL of dNTPs (10 mM each dNTP), 0.2 µL of each primer for duplex 1, 0.07 µL (BR44) and 0.12 µL (BR45) for duplex 2, 0.08 µL (BR47) and 0.1 µL (BR49) for duplex 3 (all primers at 10 µM), 0.5 U of Taq polymerase (GoTaq, Promega), approximately 50 ng of DNA extract, and the corresponding difference with Milli-Q water to complete the volume for each duplex. The temperature profile consisted of initial denaturation at 94 °C (2 min); followed by 32 cycles of denaturation at 94 °C (30 s), annealing at 62 °C (duplex 1 and 2) or 60 °C (duplex 3) (35 s), elongation at 72 °C (40 s); and a final elongation at

72 °C (30 min) prior to rapid cooling to 4 °C. Every PCR reaction run performed included a negative control reaction, containing all of the reagents except for the DNA template.

The PCR products were electrophoretically separated and visualized in an ABI 3130 (Applied Biosystems) automated sequencer. Allele sizes were defined in relation to the standard size marker GeneScan™-500 ROX™ (Applied Biosystems) (primers Pcor) or GeneScan-500(-250) ROX™ (Applied Biosystems) (primers BR) using analyses from the program PEAKSCANNER™ version 1.0 (Applied Biosystems). Program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) was used to detect evidence for null alleles in the data sets for each locus.

Comparison between geographic samples

First, the departure from HWE expectations was tested for all the individuals together (284 specimens spread over the six locations studied) to test whether the hypothesis of a single global genetic unit could be rejected using the f estimator (Wright's F-statistics, 1978; Weir and Cockerham 1984) of the endogamy index F_{IS} implemented in the program GENETIX version 4.05.2 (Belkhir et al. 1996–2004). If departure from HWE expectations occurs, a population structure involving a Wahlund effect, among other factors, will give rise to deficiency of heterozygotes and a positive value of f .

Then, tests of departure from HWE for each geographic sample (f estimator) and the degree of genetic differentiation among them, using Weir and Cockerham's (1984) θ , a widely-used analogue of the fixation index F_{ST} (Wright's F-statistics, 1978), were implemented in the program GENETIX version 4.05.2 (Belkhir et al. 1996–2004). The significance of the f and θ estimators was calculated using 1,000 random permutations from the original data.

Identification of panmictic populations (cluster analysis)

As *B. rousseauxii* undergoes great migrations throughout its life cycle, and samples were collected in different years and seasons, each geographic sample could be constituted of individuals belonging to different genetic units. Hence, panmictic units were investigated without a priori categorization of individuals. Population genetic structure then was investigated in the total sample using a Bayesian approximation. This approach consisted of a cluster analysis on various levels of hierarchy with Bayesian Analysis of Population Structure (BAPS) version 5.2 (Corander et al. 2003, 2004) and STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). The BAPS algorithm is

known to correctly identify subpopulations with F_{ST} values as low as 0.02–0.03, with accuracy similar to STRUCTURE, one of the most common programs for population analysis (e.g., Latch et al. 2006).

The procedure in BAPS for estimating the optimal number of subpopulations (K , groupings of individuals) with the highest probability consisted of executing 10 independent runs of the program with a vector made up of 10 repetitions of each value for $K = 2–8$. Probability was computed on the basis of the best partitions that were visited during all runs, and the number of clusters inferred (K) was evaluated by the modal value of the replicates. Once the most probable numbers of inferred clusters were identified, separate runs consisting of 100 and 1,000 repetitions were executed. This procedure helped choosing the best partition according to the logarithm of the highest marginal probability [Log(mp)] value.

The assignments in STRUCTURE consisted of a burn-in of 5×10^5 iterations followed by a Markov Chain Monte-Carlo procedure of 10^6 steps. STRUCTURE and BAPS try to group individuals into clusters that conform to the best HWE and linkage equilibrium. Average posterior probabilities, were calculated from 10 independent runs for $K = 1$ to $K = 8$. The estimation of the most probable K (number of biological subgroups in the entire sample) was identified following recommendations by the Evanno et al. (2005) method through STRUCTURE HARVESTER (Earl and von Holdt 2012). The admixture ancestry model and correlated allele frequencies were used, as this configuration is considered the best (Falush et al. 2007).

The most probable K value was chosen by testing departure from HWE in the cluster assignments performed by BAPS and STRUCTURE. That is, the best assignment provided clusters having the smallest departure from Hardy–Weinberg equilibrium. Program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004), was used to detect evidence for null alleles in clusters.

Intra-population genetic diversity, test of HWE and linkage disequilibrium

The genetic variability within each identified cluster was quantified and evaluated with standard descriptive statistics: number of alleles per locus (A), unbiased heterozygosity (H_{nb} —Nei 1978), observed heterozygosity (H_{obs}). Additionally, the program GENALEX version 6.4 (Peakall and Smouse 2006) was used to obtain the distribution of private alleles for each identified cluster. Tests for genotypic disequilibrium among all locus pairs (independence among loci), according to Black and Krafur's (1995) algorithm, also was carried out with GENETIX program. Linkage disequilibrium in a subdivided population can result from a natural epistatic selection within subpopulations creating a

frequency correlation among the linked alleles (Lewontin 1974). However, with limited migration among subpopulations and small effective size, random drift can increase linkage disequilibrium frequency. To differentiate between random and selective equilibrium effects, Ohta (1982a, b) devised a method to divide the variance of a coefficient inferred from two loci in disequilibrium into components that occur within and among subpopulations. Ohta's method is based on the hypothesis that when selection produces specific combinations of alleles, they should appear consistently among the subpopulations. Variability in the observed frequency of allele combinations among subpopulations must therefore be smaller under selection than under drift (Black and Krafus 1995). Five 'D' statistics describe the variance in observed and expected frequencies of allelic combinations, and such statistics are calculated for pairs of loci. The presentation of the results follow Ohta's notation, and the statistics are indicated with the subscripts I (individuals), S (subpopulations), and T (total population).

Differentiation among genetic populations

To determine the differentiation among inferred clusters, the variance of allelic identity was used [F -statistics, F_{ST} ; measured by the analogue Weir and Cockerham 's θ (1984)]. The work was carried out using F_{ST} values rather than R_{ST} values which consider allelic size variation (Slatkin 1995) due to the better estimate performance of F_{ST} when divergence among units is expected to be low (Balloux and Goudet 2002). The θ was estimated as for the geographical samples.

Results

Comparison of geographic samples

The evaluation of the multilocus estimator f value (9 loci, 129 alleles) for the global sample (284 individuals, six locations from Upper Madera and Western Amazon) indicated a strong and significant deviation from HWE ($f = 0.031$; $p < 0.001$). In the single-locus analysis, BR45 was the only locus that showed a significant departure from HWE. However, the observed deviation was maintained even when a new global analysis was performed on eight loci, excluding BR45 ($f = 0.023$; $p = 0.021$). Therefore, the overall deviation from HWE depended on a multiple-loci combination, and not only on the information contained at one single locus.

Considering each geographic sample, Cachuela Esperanza—CE ($f = 0.095$, $p = 0.001$) and Rurrenabaque—RU ($f = 0.042$, $p = 0.034$) were the only samples with a

significant departure from HWE (Table 2). At the regional scale, HWE was rejected in the Upper Madera sample (220 individuals, five geographic samples, $f = 0.033$, $p = 0.001$), but not in the Western Amazon sample (64 individuals, one regional sample, $f = -0.011$, $p = 0.724$).

The regional comparison revealed a significant differentiation ($\theta = 0.018$, $p = 0.010$) between the Western Amazon and Upper Madera samples. Pair-wise θ comparisons showed that the sample from the Western Amazon (Iquitos—IQ) was significantly different ($p < 0.001$) from each of the local geographic samples from the Upper Madera (Table 1), and indicated a significant differentiation between Villa Bella—VB and Puerto Villarroel—PV ($\theta = 0.009$, $p = 0.029$) within the Upper Madera.

Identification of panmictic populations (cluster analysis)

Given the significant overall deviation from HWE ($p < 0.001$), more specifically in the Upper Madera ($p < 0.001$) and the migratory behaviour of *B. rousseauxii*, an admixture of populations was likely in our geographic sampling collection, resulting in a Wahlund effect (Hartl and Clark 1997). Therefore, STRUCTURE and BAPS analyses were carried out to find the most probable reproductive units (clusters). Whereas the estimator θ differentiated the regional sample of Iquitos from all the others, STRUCTURE did not identify different units within the Western Amazon (Iquitos) and Upper Madera. On the other hand, three clusters were identified by BAPS ($K = 3$, marginal $-9,231.41$ and posterior 0.97 probabilities), with a distribution of allelic frequencies and genetic parameters (Hnb, Hobs, F_{IS}) presented in Table 2. Consequently, considering the overall deviation from HWE for the whole data set, the BAPS assignment ($K = 3$) was considered as the most biologically plausible partition, as a reproductive unit is defined by panmixia in a sexual reproducing species.

This partition into three units consisted of two large clusters of 83 (Cluster 1) and 184 (Cluster 2) individuals, and a smaller one of 17 individuals (Cluster 3), with a specific geographic distribution (Fig. 1). Individuals assigned to the small Cluster 3 were basically the same (apart for two individuals) when analyses were performed with 100 and 1,000 replicates, respectively, for $k = 3$. No correlation was observed among cluster conformation and standard length of the individuals ($p > 0.05$).

According to population genetics theory, loci showing large F_{ST} values between populations should be the loci for which F_{IS} are highest in population mixtures (Wahlund effect). In a first step, clusters 1 and 2 (83 and 184 individuals, respectively) were compared, computing θ (F_{ST}) values between the two clusters at each locus and the F_{IS} values combining the two clusters at each locus, and then

Table 1 Pair-wise θ (F_{ST}) values (Weir and Cockerham 1984) for the geographic samples of Dorado (*Brachyplatystoma rousseauxii*) from the Western Amazon and Upper Madera

	IQ (65)	VB	CE	PM	RU	PV
IQ						
VB (20)	0.020 ($p = 0.000$)					
CE (50)	0.023 ($p = 0.000$)	-0.004 ($p = 0.825$)				
PM (50)	0.021 ($p = 0.000$)	-0.002 ($p = 0.616$)	-0.001 ($p = 0.525$)			
RU (49)	0.015 ($p = 0.000$)	0.005 ($p = 0.121$)	0.000 ($p = 0.425$)	0.002 ($p = 0.242$)		
PV (50)	0.0170 ($p = 0.000$)	0.009 ($p = 0.029$)	0.004 ($p = 0.070$)	0.001 ($p = 0.377$)	0.001 ($p = 0.241$)	

Numbers in parenthesis next to locality abbreviations represent the size sample

VB Villa Bella, CE Cachuela Esperanza, PM Puerto Maldonado, RU Rurrenabaque, PV Puerto Villaruel, IQ Iquitos

Significant values ($p < 0.05$) highlighted in bold. Negative θ values represent zero

plotting the F_{IS} and θ (F_{ST}) values across the nine loci analysed. No correlation was observed ($r^2 = 0.086$). However, as the test is very sensitive to the mixture proportions (Waples 2011), in a second step the analysis was run again after having randomly eliminated 101 individuals in Cluster 2 in order to compare two clusters of equal size (83 individuals each). In this case also, no correlation ($r^2 = 0.00005$) was observed. These results emphasize the fact the information provided by each locus is not enough to explain the structuring in clusters or to explain the deviation from HWE when clusters are in admixture, whereas the combination of multi-loci allelic frequencies explains both the deviation from HWE in the Upper Madera sample and its structuring in clusters.

Cluster 1 was not observed in the Western Amazon samples (Iquitos), whereas it was identified in all five locations from the Upper Madera (Bolivian Amazon sites + Puerto Maldonado in Peru). Individuals from the upper-most locations in the basin (Rurrenabaque and Puerto Villaruel) were in larger proportion in Cluster 2 than in clusters 1 and 3. Cluster 2, the largest, was distributed both in the Upper Madera and in the Western Amazon. It included all but one of the individuals from the Western Amazon (Iquitos) system (64 out of 65), and a substantial proportion (45 %) of the fish caught in the lower reaches of the Upper Madera River (Villa Bella, Cachuela Esperanza and Puerto Maldonado). Finally, Cluster 3, the smallest and most differentiated, included individuals from all sampled locations, although just one specimen from the Western Amazon (Iquitos) system was assigned to this group (Fig. 1).

The genetic diversity within each cluster was moderate (Table 2), with an average observed heterozygosity (H_{obs}) ranging from 0.66 (Cluster 3) to 0.71 (Cluster 2), and numbers of alleles per locus ranging from 6.67 (Cluster 3) to 13.11 (Cluster 2). Cluster 2 had the highest number of unique alleles (39) and Cluster 1 the lowest (4). Generally, the frequencies of these alleles were low (<6 %). The locus

with the highest number of private alleles per cluster was Pcor1, with 14 private alleles in Cluster 2.

Theta values between clusters 1–2, 1–3 and 2–3 were 0.028, 0.043 and 0.038, respectively.

Linkage disequilibrium analysis between pairs of loci showed a non-systematic pattern, where D_{ST}^2 (0.006–0.041) > D_{IS}^2 (0.002–0.009) and D_{IS}^2 (0.016–0.095) > D_{ST}^2 (0.001–0.004) (minimum and maximum values of variance components of disequilibrium coefficients), consistent with the hypothesis that variation within loci depends more on genetic drift than on selection (Ohta 1982a, b).

Discussion

Clear evidence of genetic differentiation between regional samples of *B. rousseauxii* from the Upper Madera and the Western Amazon was observed, and therefore the hypothesis of a single panmictic population across the Amazon basin suggested by Batista (2010) should be rejected. Furthermore, we found evidence for three clusters of multilocus genetic variation, suggesting three differentiated populations within the Upper Madera and Western Amazon systems.

Although Latch et al. (2006) suggested that the accuracy of BAPS is as good as STRUCTURE at low levels of differentiation (F_{ST} values as low as 0.02–0.03), BAPS was later shown to perform better than STRUCTURE in identifying subsets (clustering) (e.g., Corander et al. 2008; Wilkinson et al. 2011; Neophytou 2014). This was also the case for *B. rousseauxii*, where BAPS identified panmictic clusters with low levels of differentiation.

The degree of differentiation observed with microsatellites between the clusters was low relative to that reported in other groups of migratory fishes in the Neotropics, as for example *Pseudoplatystoma* (see Abreu et al. 2009: $\theta = 0.229$; Pereira et al. 2009: $\theta = 0.034$ –0.164) and *Piaractus* (Calcagnotto and DeSalle 2009: $\theta = 0.052$ –0.065)

Table 2 Average heterozygosity at nine microsatellite loci, and values of the estimator f of the endogamy index F_{IS} calculated with multilocus data, for the three Dorado (*Brachyplatystoma rousseauxii*) clusters (Clus) as identified by BAPS and geographic samples in the Upper Madera and Western Amazon (Bolivian and Peruvian Amazon)

	Clus 1	Clus 2	Clus 3	VB	CE	PM	RU	PV	IQ	Global
N	83	184	17	20	50	50	49	50	65	284
<i>Pcor1</i>										
A	11	26	11	12	20	12	19	15	24	32
H nb	0.735	0.870	0.832	0.830	0.821	0.802	0.848	0.863	0.892	0.847
H obs	0.759	0.875	0.824	0.800	0.760	0.860	0.816	0.880	0.892	0.842
F_{IS}	-0.032	-0.005	0.011	0.037	0.076	-0.073	0.038	-0.019	-0.001	0.007
p				0.486	0.137	0.908	0.315	0.476	0.588	0.347
<i>Pcor2</i>										
A	8	15	5	7	11	10	13	11	12	17
H nb	0.700	0.768	0.652	0.830	0.821	0.802	0.848	0.863	0.892	0.745
H obs	0.663	0.772	0.647	0.800	0.760	0.860	0.816	0.880	0.892	0.732
F_{IS}	0.053	-0.005	0.008	0.312	0.017	0.078	-0.023	-0.095	-0.012	0.017
p				0.013	0.477	0.205	0.460	0.116	0.515	0.282
<i>Pcor7</i>										
A	5	9	7	5	6	7	6	5	6	9
H nb	0.442	0.259	0.415	0.554	0.364	0.353	0.261	0.188	0.327	0.326
H obs	0.410	0.266	0.412	0.550	0.320	0.360	0.245	0.200	0.339	0.313
F_{IS}	0.073	-0.029	0.009	0.007	0.123	-0.021	0.062	-0.066	-0.035	0.039
p				0.665	0.965	0.657	0.859	1.000	0.743	0.106
<i>BR37</i>										
A	6	11	4	5	5	6	6	8	11	12
H nb	0.638	0.689	0.668	0.623	0.685	0.653	0.712	0.666	0.646	0.673
H obs	0.627	0.701	0.353	0.450	0.640	0.640	0.633	0.680	0.739	0.655
F_{IS}	0.017	-0.018	0.480	0.283	0.066	0.021	0.112	-0.021	-0.144	0.027
p				0.055	0.269	0.470	0.131	0.466	0.022	0.210
<i>BR38</i>										
A	15	20	9	11	16	16	16	17	18	20
H nb	0.895	0.917	0.854	0.899	0.898	0.901	0.910	0.912	0.916	0.913
H obs	0.855	0.924	0.824	0.800	0.860	0.900	0.918	0.940	0.908	0.898
F_{IS}	0.044	-0.007	0.037	0.112	0.043	0.001	-0.010	-0.032	0.009	0.016
p				0.152	0.230	0.568	0.545	0.332	0.337	0.148
<i>BR44</i>										
A	7	9	7	6	9	8	9	9	9	9
H nb	0.791	0.799	0.711	0.790	0.809	0.807	0.806	0.801	0.774	0.800
H obs	0.121	0.079	0.029	0.900	0.680	0.820	0.755	0.780	0.769	0.771
F_{IS}	0.040	0.007	0.177	-0.144	0.161	-0.017	0.064	0.026	0.006	0.036
p				0.149	0.016	0.492	0.185	0.423	0.511	0.091
<i>BR45</i>										
A	9	12	6	7	9	10	9	8	11	12
H nb	0.823	0.843	0.804	0.815	0.829	0.857	0.846	0.835	0.825	0.844
H obs	0.783	0.772	0.765	0.750	0.680	0.780	0.816	0.840	0.769	0.775
F_{IS}	0.049	0.085	0.050	0.082	0.181	0.090	0.036	-0.006	0.068	0.082
p				0.300	0.007	0.069	0.326	0.538	0.121	0.000
<i>BR47</i>										
A	4	7	5	4	6	6	7	5	6	8
H nb	0.583	0.496	0.658	0.530	0.585	0.554	0.555	0.583	0.479	0.550
H obs	0.554	0.467	0.765	0.500	0.460	0.560	0.469	0.600	0.462	0.507

Table 2 continued

	Clus 1	Clus 2	Clus 3	VB	CE	PM	RU	PV	IQ	Global
F_{IS}	0.050	0.057	-0.169	0.057	0.216	-0.012	0.156	-0.029	0.036	0.079
p				0.364	0.025	0.551	0.069	0.470	0.423	0.025
<i>BR49</i>										
A	10	9	6	9	7	8	9	10	8	10
H nb	0.822	0.776	0.672	0.863	0.827	0.811	0.816	0.799	0.754	0.815
H obs	0.843	0.815	0.765	0.950	0.820	0.840	0.816	0.800	0.785	0.820
F_{IS}	-0.026	-0.051	-0.143	-0.104	0.009	-0.037	-0.001	-0.002	-0.041	-0.007
p				0.211	0.507	0.361	0.610	0.572	0.329	0.549
<i>Multilocus</i>										
A	8.3	13.1	6.7	7.3	9.9	9.2	10.4	9.8	11.7	129
H nb	0.714	0.713	0.696	0.736	0.723	0.719	0.719	0.715	0.710	0.724
H obs	0.695	0.710	0.660	0.689	0.656	0.716	0.689	0.731	0.716	0.702
F_{IS}	0.027	0.005	0.054	0.066	0.095	0.005	0.042	-0.023	-0.009	0.031
p				0.942	0.000	0.425	0.034	0.858	0.705	0.000

N number of individuals, *A* Number of alleles, *H nb* heterozygosity calculated without bias (Nei 1978), *H obs* observed heterozygosity, *VB* Villa Bella, *CE* Cachueta Esperanza, *PM* Puerto Maldonado, *RU* Rurrenabaque, *PV* Puerto Villarroel, *IQ* Iquitos
Significant values ($p < 0.05$) highlighted in bold

Table 3 Comparisons of Weir and Cockerham's (1984) θ (F_{ST}) values (for microsatellites) among clusters of Dorado (*Brachyplatystoma rousseauxii*) defined by BAPS in the Upper Madera and Western Amazon (Bolivian and Peruvian Amazon), and those for other freshwater, brackish and marine migratory fish species

Order	Family	Species	Environment	Behaviour	Loci	θ (F_{ST}) ($p < 0.05$)	Reference
Siluriformes	Pimelodidae	<i>Brachyplatystoma rousseauxii</i>	Continental	Migratory-amphidromous	9	0.028–0.043	Present study
Siluriformes	Pimelodidae	<i>Pseudoplatystoma corruscans</i>	Continental	Migratory-potamodromous	7	0.034–0.164	Pereira et al. (2009)
Siluriformes	Pimelodidae	<i>Pseudoplatystoma reticulatum</i>	Continental	Migratory-potamodromous	7	0.229	Abreu et al. (2009)
Characiformes	Serrasalminidae	<i>Piaractus mesopotamicus</i>	Continental	Migratory-potamodromous	8	0.052–0.065	Calcagnotto and DeSalle (2009)
Cypriniformes	Cyprinidae	<i>Coreius guichenoti</i>	Continental	Migratory-potamodromous	11	0.022–0.047	Zhang and Tan (2010)
Cypriniformes	Balitoridae	<i>Barbatula barbatula</i>	Continental	Migratory-potamodromous	5	0.010–0.110	Barluenga and Meyer (2004)
Salmoniformes	Salmonidae	<i>Salmo salar</i>	Marine, brackish, continental	Migratory-anadromous	8	0.027–0.072	McConnell et al. (1997)
Perciformes	Scombridae	<i>Thunnus thynnus</i>	Marine	Migratory-oceanodromous	9	0.009	Carlsson et al. (2004)
Perciformes	Scombridae	<i>Thunnus thynnus</i>	Marine	Migratory-oceanodromous	8	0.005–0.012	Carlsson et al. (2007)
Gadiformes	Gadidae	<i>Gadus morhua</i>	Marine	Migratory-oceanodromous	10	0.0013–0.005	Knutsen et al. (2003)
Gadiformes	Gadidae	<i>Gadus morhua</i>	Marine	Migratory-oceanodromous	13	0.002–0.006	Knutsen et al. (2011)
Anguilliformes	Anguillidae	<i>Anguilla anguilla</i>	Marine	Migratory-catadromous	22	0.005–0.015	Pujolar et al. (2011)
Carchariniformes	Carcharinidae	<i>Carcharinus limbatus</i>	Marine, brackish	Migratory-amphidromous	8	0.004–0.067	Keeney et al. (2005)

(Table 3). Nevertheless, these values were equal or much greater (up to ten times) than those observed for other continental and marine migratory species with low F_{ST} values that nevertheless reflect a biological reality (e.g. *Salmo salar*—McConnell et al. 1997: $\theta = 0.027$ – 0.072 ; *Gadus morhua*—Knutsen et al. 2011: $\theta = 0.002$ – 0.006) (Table 3). It can therefore be considered that our results indicate the existence of more than one genetic unit, and probably at least three in the Amazon.

Structured populations

The presence of *B. rousseauxii* along the main channel of the Amazon and its main tributaries has raised speculation that commercial fisheries exploit a single stock that moves continuously between the headwaters and the estuary (Barthem and Goulding 1997, 2007). This hypothesis recently was asserted by Batista (2010), who analysed 483 individuals from 13 locations using eight microsatellites. This study focused on the main channel of the Solimões-Amazon, but also considered several tributaries, including the Madera River (60 specimens from four localities) and the Madre de Dios River (tributary of the Madera, with 43 specimens from Puerto Maldonado). In spite of this overlap with our sampling areas, Batista (2010) inferred general panmixia over the whole *B. rousseauxii* distribution area, albeit without giving any value for their departure from HWI nor for the overall F_{IS} . On the contrary, our work revealed the existence of three clusters, two of them principally localized in the Upper Madera (Cluster 1 and Cluster 3). Furthermore, the differentiation between the Upper Madera and the Western Amazon samples was strong.

The discrepancy between the results of the present study and those of Batista (2010) may lie in the numbers and geographic origins of the samples examined from the Upper Madera basin. Batista (2010) analyzed mainly samples from the Amazon River's main channel, and considered 103 samples from two locations in the Upper Madera basin (43 from the Madre de Dios and 60 from Porto Velho), which represented less than half our sampling number. BAPS analyses revealed that Cluster 1 appears to be exclusive to the Upper Madera River, whereas the ubiquitous Cluster 2 (largest in number) is dominant in the Iquitos (Ucayali-Amazon) sample (only one out of 64 individuals did not belong to Cluster 2), and Cluster 3, the most differentiated, consists of a small number of individuals, all but one (from Iquitos) belonging to the Upper Madera. The degree of differentiation observed between the three clusters was weak ($\theta = 0.028$ – 0.043), albeit significant, and therefore its signal in the total grouping is low. It is therefore possible that the signal from clusters 1 and 3 (the least abundant) was not detected in Batista (2010)'s sampling, which contained only a

small number of individuals from the Upper Madera basin. Additionally, the ubiquitous and large Cluster 2, predominant in the Iquitos samples (all individuals but one), may also be numerically dominant along the main channel of the Amazon River (Peru and Brazil) and in the lower reaches of its main tributaries, masking the signal of other, less abundant populations, such as clusters 1 and 3, which may be present in the lower Amazon (below the confluence with the Madera), given the migratory life cycle of the species. The observation that two of the clusters identified in the Upper Madera are absent, or scarce, in the Upper Amazon (Western Amazon) suggests the existence of a homing behaviour at the scale of large sub-basins (Madera vs. Amazon). This homing hypothesis, however, requires further evidence, including use of complementary methodologies such as mark-recapture, isotopic signatures on otoliths or telemetry. Alternatively, a potential explanation for the population structure in the Upper Madera is the co-existence of more than one life-history strategy in the species. *B. rousseauxii* could be composed of long-distance migrants, as suggested by earlier work, and by one or more populations that migrate shorter distances or residualize within river systems. Co-existence of multiple life-history strategies with residualized populations have been shown in other migratory fish species, especially salmonids, when migration leads to high mortality, when migration is cut off or when various factors act synergistically to create complex patterns of movement within populations (Wood and Foote 1996; Chapman et al. 2011, 2012; Brodersen et al. 2014). Hence Clusters 1 and 3 could correspond to more resident populations and Cluster 2 to a long-distance migratory population. Testing this hypothesis would require the sampling and genetic characterization of spawning assemblages.

The existence of three distinct clusters (genetic units) in a same geographical area suggests that differential reproductive behaviour among the three populations occurs either by temporal and/or spatial segregation. Breeding seasons vary among tributaries in the Upper Madera: between October and December, with a peak in October in the Upper Madre de Dios (Cañas and Pine 2011; Cañas and Waylen 2011), between February and May in the Upper Mamoré River (Van Damme et al. 2011a), whereas females with ripe gonads were observed from October to May in the Upper Beni River (pers. obs. F. Duponchelle, Institut de Recherche pour le Développement—France, unpublished data). We also know that *B. rousseauxii* larvae are present almost year-round in the Madera (R. Leite, Instituto Nacional de Pesquisas da Amazônia—Brazil, pers. com.), and so are ripe adults in specific tributaries (R. Barthem, Museu Paraense Emílio Goeldi—Brazil, and M. Goulding, consultant of Wildlife Conservation Society—USA, pers. com.). Taking into account our biological knowledge of the species, the presence of three distinct genetic populations

in admixture in every sampled tributary may be explained either by the fact that individuals from these populations would reproduce at the same places but at different periods (temporal segregation hypothesis) with some overlap (gene flow), or may reproduce only in specific tributaries (spatial segregation hypothesis associated with a homing behaviour among the Upper Madera tributaries). Most known cases of homing are associated with some degree of straying, which could explain the presence of an individual from Cluster 3 in the Western Amazon. Clearly, further studies are needed to shed light on these two, not mutually exclusive, hypotheses.

Additional biological information that supports the existence of several populations in the Amazon basin is the difference in the maximum size observed between the Upper Amazon, where specimens of 150 cm SL are common (García et al. 2009a for the Peruvian Amazon and Agudelo et al. 2013 for the Colombian Amazon), and the Upper Madera, where specimens above 130 cm SL are rarely caught (Van Damme et al. 2011a, Carvajal-Vallejos, pers. obs.). This difference is further emphasized by the fact that fishery exploitation, which decreases the maximum size of exploited populations (Rochet and Trenkel 2003), borders on over-exploitation for this species in the Peruvian (García et al. 2009a, b) and Colombian Amazon (Agudelo et al. 2013), whereas it remains relatively weak in the Bolivian Amazon (Van Damme et al. 2011a).

Conservation implications

Acknowledging the existence of different populations will have a substantial impact on the strategies for conservation and management of this species in the Amazon Basin, which is threatened to varying degrees by overfishing, pollution, habitat destruction and dam construction in the five countries (Finer and Jenkins 2012; Castello et al. 2013).

Hydroelectric impoundments in particular appear to be the most immediate threat in the Madera River, where two dams recently were constructed at the Jirau and Santo Antônio rapids in Brazil, and several others are planned (Finer and Jenkins 2012). One of these two dams (Santo Antônio) provides a specific by-pass system for the upstream migration of fishes. Although its effectiveness is still unknown for Dorado, this by-pass system seems very efficient for several species formerly restricted to the downstream portion of the Madera below the Santo Antônio rapids close to Porto Velho (Brazil): recently, fishermen from the Bolivian Amazon have reported the captures of species never seen before (Carvajal-Vallejos, pers. obs). Although the dams may not be preventing the

upstream migration of adult *B. rousseauxii*, a major concern remains for the larvae and juveniles. The downstream migration of young stages is likely to be seriously affected by the dams and associated reservoirs, depleting or hampering the recruitment of this species from the Madera (Molina 2011b). Bolivian fishermen are also reporting unusually small sizes for several otherwise big catfish species (Carvajal-Vallejos, pers. obs), suggesting that juveniles, which were drifting downstream to the estuary before, are now unable to pass through the dam and are forced to grow in the Upper Madera. Targeted monitoring of the effects of the dams and the by-pass system on the upstream and downstream movements of the Madera ichthyofauna is needed.

As the Madera contributes nearly one-fifth of the Amazon's water balance and to about half its sediment load (Goulding et al. 2003), it appears reasonable to hypothesize that the Madera might also represent a similar proportion (>20 %) of the Amazon recruitment for *B. rousseauxii*. Moreover, as shown by our results, at least one population seems to be present exclusively in the Madera basin, which means that reduced recruitment from the Madera would translate into a lower return rate of adults to the spawning grounds, which ultimately could lead to a loss of genetic variability and decreased landings. A reduction of the landings could provoke a collapse of the fisheries for Dorado, as well as other migratory species in the Upper Madera River Basin (mainly the Bolivian Amazon and Puerto Maldonado town), having serious effects on the fisheries value chain, people's livelihoods and local food security.

While the hydroelectric projects are at an advanced stage of construction or completed in some cases, very little is known about their effect on this species. It is urgent that more data be quickly generated to complete and better understand the ecology and structure of the Dorado population throughout the basin, and confirm or refute the existence of homing or resident behaviours in the Madera basin.

Acknowledgments We gratefully acknowledge the support of the Institute de Recherche pour le Development (IRD) and the World Wildlife Foundation (WWF—Russell E. Train Education for Nature Program) for the scholarships granted to conduct this study. We extend our sincere thanks to all of the fishermen, government representatives and academic authorities in the Bolivian Amazon who kindly helped with the sampling and permits and shared their knowledge during the years of fieldwork in the Madera, Beni, Madre de Dios, Mamoré and Ichilo rivers. B. Lehner, at the Conservation Science Program, World Wildlife Fund US, generously provided the base map used in Fig. 1. Data used in this work were partly produced through the technical facilities of the labex «Centre Méditerranéen de l'Environnement et de la Biodiversité».

Conflict of interest The authors declare that they have no conflict of interest.

References

- Abreu M, Pereira LH, Vila V, Foresti F, Oliveira C (2009) Genetic variability of two populations of *Pseudoplatystoma reticulatum* from the Upper Paraguay River Basin. *Genet Mol Biol* 32:868–873
- Agudelo E, Salinas Y, Sánchez CL, Muñoz-Sosa DL, Alonso JC, Arteaga ME, Rodríguez OJ, Anzola NR, Acosta LE, Núñez M, Valdés H (2000) Bagres de la Amazonía Colombiana: Un Recurso Sin Fronteras. Instituto Amazónico de Investigaciones Científicas SINCHI. Programa de Ecosistemas Acuáticos. Bogotá, Colombia, p 252
- Agudelo E, Joven AV, Bonilla-Castillo CA, Petreter M, Peláez M, Duponchelle F (2013) Breeding, growth and exploitation of *Brachyplatystoma rousseauxii* Castelnau, 1855 in the Caqueta River, Colombia. *Neotrop Ichthyol* 11:637–647
- Alonso JC (2002) Padrão espaço-temporal da estrutura populacional e estado atual da exploração pesqueira da dourada *Brachyplatystoma flavicans*, Castelnau, 1855 (Siluriformes: Pimelodidae), no sistema Estuário-Amazonas-Solimões. Doctoral dissertation, Instituto Nacional de Pesquisas da Amazônia—INPA, Manaus (AM), Brasil
- Alonso JC, Pirker LEM (2005) Dinâmica populacional e estado actual da exploração de piramutaba e de dourada. In: Fabrè NN, Barthem RB (eds) O Manejo Da Pesca Dos Grandes Bagres Migradores: Piramutaba e Dourada No Eixo Solimões-Amazonas. Coleção Documentos Técnicos: Estudos estratégicos. IBAMA, ProVárzea, Manaus, pp 21–28
- Angelini R, Fabrè NN, Lopes da Silva-JR U (2006) Trophic analysis and fishing simulation of the biggest Amazonian catfish. *Afr J Agri Res* 1:151–158
- Araujo-Lima CARM, Goulding M (1997) So fruitful a fish: ecology, conservation, and aquaculture of the Amazon's Tambaqui. Columbia University Press, New York
- Araujo-Lima CARM, Ruffino ML (2003) Migratory fishes of the Brazilian Amazon. In: Carolsfeld J, Harvey B, Ross C, Baer A (eds) Migratory fishes of South America: biology, fisheries and conservation status. The International Bank for Reconstruction and Development, The World Bank, IDRC/2004-01-01, pp 233–302
- Balloux F, Goudet F (2002) Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. *Mol Ecol* 11:771–783
- Barluenga M, Meyer A (2004) The Midas cichlid species complex: incipient sympatric speciation in Nicaraguan cichlid fishes? *Mol Ecol* 13:2061–2076
- Barthem R, Goulding M (1997) The catfish connection. Columbia University Press, New York
- Barthem R, Goulding M (2007) Un Ecosistema Inesperado: La Amazonía Revelada Por La Pesca. Gráfica Biblios SA, Lima
- Barthem RB, Riberio MC, Petreter M (1991) Life strategies of some long-distance migratory catfish in relation to hydroelectric dams in the Amazon basin. *Biol Conserv* 55:339–345
- Barthem R, Goulding M, Forsberg B, Cañas C, Ortega H (2003) Ecología Acuática del Río Madre de Dios. Gráfica Biblios SA, Lima
- Batista J (2010) Caracterização genética da dourada—*Brachyplatystoma rousseauxii*, Castelnau, 1855 (Siluriformes: Pimelodidae) na Amazônia por meio de marcadores moleculares mitocondriais e microsatélites: subsídios para conservação e manejo. Doctoral dissertation, Instituto Nacional de Pesquisas da Amazônia—INPA, Manaus (AM), Brasil
- Batista JS, Alves-Gomez JA (2006) Phylogeography of *Brachyplatystoma rousseauxii* (Siluriformes–Pimelodidae) in the Amazon Basin offers preliminary evidence for the first case of “homing” for an Amazonian migratory catfish. *Genet Mol Res* 5:723–740
- Batista JS, Farias IP, Formiga-Aquino K, Sousa ACB, Alves-Gomes JA (2009) DNA microsatellite markers for “dourada” (*Brachyplatystoma rousseauxii*, Siluriformes: Pimelodidae), a migratory catfish of utmost importance for fisheries in the Amazon: development, characterization and inter-specific amplification. *Conserv Genet Resour* 1:5–10
- Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996–2004) GENETIX 4.05, logiciel sous Windows™ pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France)
- Black WC, Krafur ES (1995) A FORTRAN program for the calculation and analysis of two-locus linkage disequilibrium coefficients. *Theor Appl Genet* 70:491–496
- Brodersen J, Chapman BB, Nilsson PA, Skov C, Hansson L-A, Brönmark C (2014) Fixed and flexible: coexistence of obligate and facultative migratory strategies in a freshwater fish. *PLoS One* 9:e90294
- Calcagnotto D, DeSalle R (2009) Population genetic structuring in pacu (*Piaractus mesopotamicus*) across the Paraná-Paraguay basin: evidence from microsatellites. *Neotrop Ichthyol* 7:607–616
- Campos Telles MP, Garcia Collevatti R, da Costa MC, Borges Barthem R, da Silva NJ, Souza Neto AC, Felizola Diniz-Filho JAF (2011) A geographical genetics framework for inferring homing reproductive behavior in fishes. *Genetica* 139:243–253
- Cañas CM, Pine WE (2011) Documentation of the temporal and spatial patterns of Pimelodidae catfish spawning and larvae dispersion in the Madre de Dios River (Peru): insights for conservation in the Andean-Amazon headwaters. *River Res Appl* 27:602–611
- Cañas CM, Waylen PR (2011) Modelling production of migratory catfish larvae (Pimelodidae) on the basis of regional hydro-climatology features of the Madre de Dios Basin in southeastern Peru. *Hydrol Process* 26:996–1007
- Carlsson J, McDowell JR, Díaz-Jaimes P, Carlsson JEL, Boles SB, Gold JR, Graves JE (2004) Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea. *Mol Ecol* 13:3345–3356
- Carlsson J, McDowell JR, Carlsson JEL, Graves JE (2007) Genetic identity of YOY bluefin tuna from the Eastern and Western Atlantic spawning areas. *J Hered* 98:23–28
- Carvajal-Vallejos FM, Zeballos AJ (2011) Diversidad y distribución de los peces de la Amazonía boliviana. In: Van Damme P, Carvajal-Vallejos FM, Molina J (eds) Los Peces y Delfines de la Amazonía Boliviana: Hábitats, Potencialidades y Amenazas. INIA editorial, Cochabamba, Bolivia, pp 101–147
- Carvajal-Vallejos FM, Van Damme PA, Muñoz H (2011) Composición de las capturas comerciales y de subsistencia en la Amazonía boliviana. In: Van Damme P, Carvajal-Vallejos FM, Molina J (eds) Los Peces y Delfines de la Amazonía Boliviana: Hábitats, Potencialidades y Amenazas. INIA editorial, Cochabamba, Bolivia, pp 203–233
- Castello L, McGrath DG, Hess LL, Coe MT, Lefebvre PA, Petry P, Macedo MN, Renó VF, Arantes C (2013) The vulnerability of Amazon freshwater ecosystems. *Conserv Lett* 6:217–229
- Chapman BB, Brönmark C, Nilsson JÅ, Hansson L-A (2011) The ecology and evolution of partial migration. *Oikos* 120:1764–1775
- Chapman BB, Skov C, Hulthén K, Brodersen J, Nilsson PA, Skov C, Hansson L-A, Brönmark C (2012) Partial migration in fishes: causes and consequences. *J Fish Biol* 81:479–499
- Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics* 163:367–374

- Corander J, Waldmann P, Marttinen P, Sillanpää MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics* 20:2363–2369
- Corander J, Marttinen P, Sirén J, Tang J (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinform* 9:539
- Coronel JS, Maes GE, Claus S, Van Damme PA, Volckaert FAM (2004) Differential population history in the migratory catfishes *Brachyplatystoma flavicans* and *Pseudoplatystoma fasciatum* (Pimelodidae) from the Bolivian Amazon assessed with nuclear and mitochondrial DNA markers. *J Fish Biol* 65:859–868
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15
- Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour* 4:359–361
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611–2620
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567–1587
- Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol Ecol Notes* 7:574–578
- Farias I, Torrico JP, García-Davila C, Freitas MC, Hrbek T, Renno J-F (2010) Are rapids a barrier for floodplain fishes of the Amazon basin? A demographic study of the keystone floodplain species *Colossoma macropomum* (Teleostei: Characiformes). *Mol Phylogenet Evol* 56:1129–1135
- Finer M, Jenkins CN (2012) Proliferation of hydroelectric dams in the Andean Amazon and implications for Andes-Amazon connectivity. *PLoS One* 7:e35126
- García A, Tello S, Vargas G, Duponchelle F (2009a) Patterns of commercial fish landings in the Loreto region (Peruvian Amazon) between 1984 and 2006. *Fish Physiol Biochem* 35:53–67
- García A, Alonso J-C, Carvajal F, Moreau J, Nuñez J, Renno J-F, Tello S, Montreuil V, Duponchelle F (2009b) Life-history characteristics of the large Amazonian migratory catfish *Brachyplatystoma rousseauxii* in the Iquitos region, Peru. *J Fish Biol* 75:2527–2551
- Goulding M (1979) *Ecologia da Pesca no Rio Madeira*. INPA, CNPq, Manaus, Brazil
- Goulding M (1980) *The fishes and the forest*. California University Press, Berkeley
- Goulding M, Barthem R, Ferreira EJ (2003) *The Smithsonian Atlas of the Amazon*. Smithsonian Institution Press, Washington, DC
- Hartl DL, Clark AG (1997) *Principles of population genetics*, 3rd edn. Sinauer Associates, Sunderland
- Junk WJ, Soares MG, Bayley PB (2007) Freshwater fishes of the Amazon River basin: their biodiversity, fisheries, and habitats. *Aquat Ecosyst Health Manag* 10:153–173
- Kaplinski L, Anderson R, Puurand T, Remm M (2004) MultiPLX: automatic grouping and evaluation of PCR primers. *Bioinformatics* 21:1701–1702
- Keeney DB, Heupel MR, Hueter RE, Heist EJ (2005) Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the north-western Atlantic, Gulf of Mexico, and Caribbean Sea. *Mol Ecol* 14:1911–1923
- Knutsen H, Jorde PE, André C, Stenseth NCHR (2003) Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod. *Mol Ecol* 12:385–394
- Knutsen H, Olsen EM, Jorde PE, Espeland SH, André C, Stenseth NC (2011) Are low but statistically significant levels of genetic differentiation in marine fishes 'biologically meaningful'? A case study of coastal Atlantic cod. *Mol Ecol* 20:768–783
- Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conserv Genet* 7:295–302
- Lauzanne L, Loubens G, Le Guennec B (1999) Pesca y biología pesquera en el Mamoré Medio (región de Trinidad, Bolivia). *Interciencia* 15:452–460
- Lewontin RC (1974) *The genetic basis of evolutionary change*. Columbia University Press, New York, pp 273–318
- McConnell SKJ, Ruzzante DE, O'Reilly PT, Hamilton L, Wright JM (1997) Microsatellite loci reveal highly significant genetic differentiation among Atlantic salmon (*Salmo salar* L.) stocks from the east coast of Canada. *Mol Ecol* 6:1075–1089
- Molina J (2011a) Funcionamiento hidráulico del río Madera en la zona de cachuelas. In: Van Damme P, Carvajal-Vallejos FM, Molina J (eds) *Los Peces y Delfines de la Amazonía Boliviana: Hábitats, Potencialidades y Amenazas*. INIA editorial, Cochabamba, Bolivia, pp 29–50
- Molina J (2011b) Funcionamiento de componentes de las represas del río Madera en relación al paso de peces: Vertederos, turbinas y sistemas de transposición de peces. In: Van Damme P, Carvajal-Vallejos FM, Molina J (eds) *Los Peces y Delfines de la Amazonía Boliviana: Hábitats, Potencialidades y Amenazas*. INIA editorial, Cochabamba, Bolivia, pp 417–436
- Molina J, Vauchel P (2011) Régimen hidrológico del río Madera y de sus tributarios. In: Van Damme P, Carvajal-Vallejos FM, Molina J (eds) *Los Peces y Delfines de la Amazonía Boliviana: Hábitats, Potencialidades y Amenazas*. INIA editorial, Cochabamba, Bolivia, pp 3–14
- Navarro G, Maldonado M (2002) *Biografía Ecológica de Bolivia: Vegetación y Ambientes Acuáticos*. Centro de Ecología Simón I. Patiño—Departamento de Difusión. Cochabamba, Bolivia
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590
- Neophytou C (2014) Bayesian clustering analyses for genetic assignment and study of hybridization in oaks: effects of asymmetric phylogenies and asymmetric sampling schemes. *Tree Genet Genomes* 10:273–285
- Ohta T (1982a) Linkage disequilibrium due to random genetic drift in finite subdivided populations. *Proc Natl Acad Sci USA* 79:1940–1944
- Ohta T (1982b) Linkage disequilibrium with the island model. *Genetics* 101:139–155
- Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288–295
- Pereira LHG, Foresti F, Oliveira C (2009) Genetic structure of the migratory catfish *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae) suggests homing behaviour. *Ecol Freshw Fish* 18:215–225
- Petrere M, Barthem R, Agudelo E, Corrales B (2004) Review of the large catfish fisheries in the upper Amazon and the stock depletion of piraíba (*Brachyplatystoma filamentosum* Lichtenstein). *Rev Fish Biol Fish* 14:403–414
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Pujolar JM, Bevacqua D, Andreollo M, Capoccioni F, Ciccotti E, De Leo GA, Zane L (2011) Genetic patchiness in European eel adults evidenced by molecular genetics and population dynamics modeling. *Mol Phylogenet Evol* 58:198–206

- Revaldaves E, Pereira LHG, Foresti F, Oliveira C (2005) Isolation and characterization of microsatellite loci in *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae) and cross-species amplification. *Mol Ecol Notes* 5:463–465
- Rochet M-J, Trenkel VM (2003) Which community indicators can measure the impact of fishing? A review and proposals. *Can J Fish Aquat Sci* 60:86–99
- Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457–462
- Tavera G, Becerra P, Ruiz-García M, Carvajal-Vallejos FM, Van Damme P (2011) Distribución y estado poblacional del delfín boliviano (*Inia boliviensis*) en la Amazonía boliviana. In: Van Damme P, Carvajal-Vallejos FM, Molina J (eds) *Los Peces y Delfines de la Amazonía Boliviana: Hábitats, Potencialidades y Amenazas*. INIA editorial, Cochabamba, Bolivia, pp 53–63
- Torrente-Vilara G, Zuanon J, Leprieur F, Oberdorff T, Tedesco PA (2011) Effects of natural rapids and waterfalls on fish assemblage structure in the Madeira River (Amazon Basin). *Ecol Freshw Fish* 20:588–597
- Van Damme PA, Carvajal-Vallejos FM, Camacho J, Muñoz H, Coronel J (2011a) Peces migratorios de la Amazonía boliviana. In: Van Damme PA, Carvajal-Vallejos FM, Molina J (eds) *Los Peces y Delfines de la Amazonía Boliviana: Hábitats, Potencialidades y Amenazas*. INIA editorial, Cochabamba, Bolivia, pp 149–200
- Van Damme PA, Carvajal-Vallejos FM, Pouilly M, Pérez T, Molina Carpio J (2011b) Amenazas para los peces y pesquerías de la Amazonía boliviana. In: Van Damme PA, Carvajal-Vallejos FM, Molina J (eds) *Los Peces y Delfines de la Amazonía Boliviana: Hábitats, Potencialidades y Amenazas*. INIA editorial, Cochabamba, Bolivia, pp 327–365
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538
- Waples R (2011) Can evidence for spatial and/or temporal genetic heterogeneity of North Pacific minke whales be explained by different mixture fractions of the same two core stocks, or is it necessary to postulate an additional stock (s)? Publications, Agencies and Staff of the U.S. Department of Commerce. Paper 460
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution* 38:1358–1370
- Wilkinson MJ, Marshall LG, Lundberg JG, Kreslavsky MH (2010) Megafan environments in northern South America and their impact on Amazon Neogene aquatic ecosystems. In: Hoorn C, Wesselingh FP (eds) *Amazonia, landscape and species evolution: a look into the past*. Blackwell Publishing Ltd, Oxford, pp 162–184
- Wilkinson S, Haley C, Alderson L, Wiener P (2011) An empirical assessment of individual-based population genetic statistical techniques: application to British pig breeds. *Heredity* 106:261–269
- Wood CC, Foote CJ (1996) Evidence for sympatric genetic divergence of anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution* 50:1265–1279
- Wright S (1978) *Evolution and the genetics of populations: variability within and among natural populations*, vol 4. University of Chicago Press, Chicago
- Zhang F, Tan D (2010) Genetic diversity in population of largemouth bronze gudgeon (*Coreius guichenoti* Sauvage & Dabry) from Yangtze River determined by microsatellite DNA analysis. *Genes Genet Syst* 85:351–357