

## Rapid Communication

## First record of the invasive blue peacock cichlid *Cichla piquiti* Kullander and Ferreira 2006 (Cichliformes: Cichlidae) in the Paraíba do Sul river basin, south eastern Brazil

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### Abstract

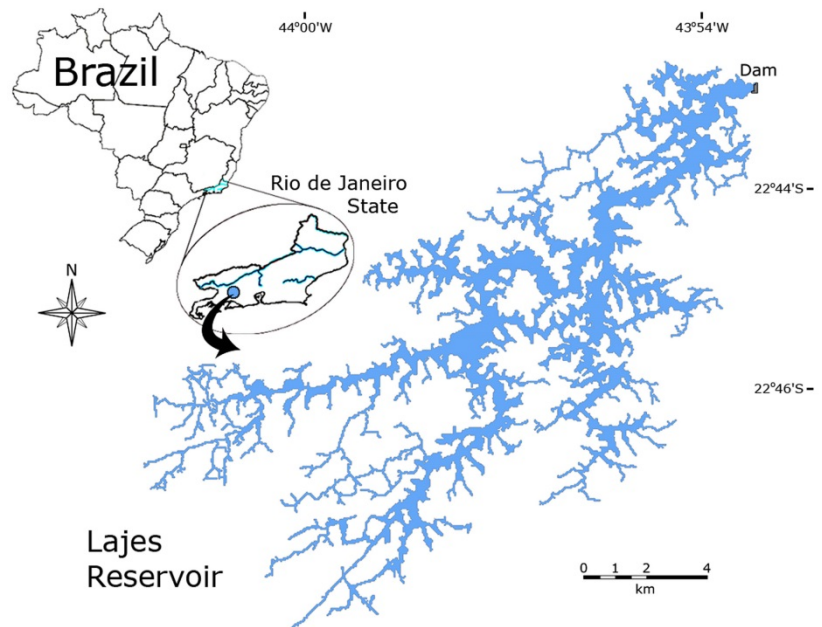
The blue peacock cichlid *Cichla piquiti* is naturally found in the Tocantins-Araguaia river basins, but non-native populations are recorded in many reservoirs of Central and South Brazil. The present study provides, through both morphological and molecular analyses, the first record of the introduction of *C. piquiti* in a large and important river basin of south eastern Brazil. Morphological characteristics together with 35 cytb and 48 CR mtDNA sequences confirmed the putative sampled fish as *C. piquiti*, although hybridisation with *C. kelberi* (i.e. another *Cichla* species introduced at Lajes reservoir in the 1950s) cannot be entirely ruled out. Two stocking events were carried out in 2005 and 2010 by affiliated anglers of a local sportfishing club to introduce *C. piquiti* into Lajes, but only the latter resulted in established populations. Since *C. piquiti* can apparently achieve higher maximum size than *C. kelberi*, it is expected that the vulnerability of native fish to predation will be higher. In addition, there is the possibility of introgressive hybridisation between the two introduced *Cichla* species, which can obscure the recognition of successive events of introduction and potentially increase the impact on native fish assemblages. Finally, *C. piquiti* has the potential to become widespread, both by passive movement along interconnected systems within the Paraíba do Sul river basin, as well as through active transfer by local anglers to other more distant systems, following a similar trend to that which apparently occurred for *C. kelberi*. Therefore, the continuous assessment of peacock cichlid populations, together with further studies on the genetic characterization of *C. piquiti*, have great ecological relevance for the early detection of the spread of this invasive fish beyond the Paraíba do Sul river basin and throughout Brazilian territory.

**Key words:** Introduction, alien fish, peacock bass, morphological traits, molecular analysis, Neotropical

### Introduction

Peacock cichlids (*Cichla* spp.) are prized game fish that have been continuously introduced into freshwater systems around the world, especially in South, Central and North Americas (Zaret and Paine 1973; Nelson 1994; Shafland 1996; Santos et al. 2011; Espínola et al. 2015) but also in Africa and Asia (Concepcion and Nelson 1999; Hickley et al. 2008; Yeo and Chia 2010; Rahim et al. 2013; Guerrero

2014). The blue peacock cichlid *Cichla piquiti* Kullander and Ferreira 2006 is naturally found in the Tocantins-Araguaia river basins, Brazil, but non-native populations are commonly recorded in many reservoirs of the Paraná and Paranaíba river basins (Vieira et al. 2009; Luiz et al. 2011). In contrast to yellow peacock cichlids, in which species identification is difficult, *C. piquiti* is easily distinguished from its congeners by its blue-tone body and the presence of five dark vertical bars below the dorsal fin.



**Figure 1.** Geographic location of Lajes reservoir, showing its position in the upper stretches of the Paraíba do Sul river basin.

Some characteristics exhibited by *C. piquiti*, also shared by other peacock cichlids, are associated with its high invasibility in Brazilian reservoirs, such as biparental care (Muñoz et al. 2006), opportunistic piscivorous habit (Jepsen et al. 1997) and adaptations to thrive in lentic environments (Resende et al. 2008). In addition, *C. piquiti* can grow up to 700 mm in total length, ranking amongst the biggest *Cichla* species (Kullander and Ferreira 2006), thus probably heightening the predation effects on native prey.

Non-native invasive species trigger numerous adverse impacts, and are recognized as one of the major threats to natural ecosystems and biodiversity (Simberloff 2003; Clavero and García-Berthou 2005). The introduction of *C. kelberi* in the 1950s in Lajes Reservoir, Paraíba do Sul river basin, is among the oldest episodes of peacock cichlid translocation outside its native range and is also the first non-native peacock cichlid population recorded in south eastern Brazil (Santos et al. 2008). The predation effects of *C. kelberi* over ~70 years since its introduction is considered as the major cause of the low richness and abundance of native fish species in this 30km<sup>2</sup> oligotrophic reservoir, situated in the upper stretches of the Paraíba do Sul river basin (Araújo and Santos 2001; Santos et al. 2001, 2008). More recently, Marques et al. (2016) suggested through molecular analysis that *C. kelberi* probably dispersed from Lajes reservoir to other impoundments in the Paraíba do Sul river, thus increasing the impacts at a regional scale.

The present study provides the first record of the introduction of *C. piquiti* in the Paraíba do Sul river basin, which encompasses areas from the three most populated cities in Brazil and accounts for the major water supply source for Rio de Janeiro State. Here, molecular analyses of mtDNA markers are applied together with morphological measurements to the identification of *C. piquiti*. The major implications of *C. piquiti* introduction at local and regional scales are also discussed.

## Material and methods

### Study site

Lajes reservoir (22°42'N–43°53'W; 22°50'N–44°05'W; Figure 1) is a 30km<sup>2</sup> impoundment located 415 m above mean sea level in Paraíba do Sul river basin, the largest river in Rio de Janeiro State, south eastern Brazil. This reservoir was filled between 1905 and 1908 mainly for hydroelectric purposes, damming streams and diverting small rivers of the East Hydrographic Basin. Lajes reservoir also contributes as the major source of drinking water to the Rio de Janeiro metropolis (~12.3 million people; IBGE 2016), since its pumped waters flow to Guandu river (Araújo and Santos 2001). Lajes is oligotrophic (i.e. <20 mgL<sup>-1</sup> total P; <400 mgL<sup>-1</sup> total N) and largely surrounded by well-preserved stretches of Atlantic Rainforest reserve, but it has experienced recent trends of nutrient enrichment due

to increased anthropogenic activities (i.e. diffuse pollution) and high water retention time (286 days) (Guarino et al. 2005; Branco et al. 2009; Santos et al. 2011). Because of vegetation removal prior to reservoir filling and large water level fluctuations (up to 12 m per year) from dam operational routine, Lajes reservoir is structurally homogeneous, lacking aquatic macrophytes and other natural submerged structures (Santos et al. 2008). The non-native peacock cichlid *C. kelberi* was introduced into the reservoir at the 1950s to control forage fish overpopulation and improve local angling activities (Santos et al. 2001). Despite the probable adverse impacts on the indigenous fish species, legal management actions to protect *C. kelberi* (i.e. minimum capture size, spawning season protection and bag limits) surprisingly persist in Lajes reservoir.

#### *Sampling and morphological identification*

Samples of introduced peacock cichlids were caught by angling (using artificial baits) from April to June of 2014, 2015, and 2016 in Lajes reservoir, Paraíba do Sul River basin. All specimens were euthanized in ice, measured (total and standard length; mm), and weighed (g). The following measurements were performed in all individuals (mm), according to Kullander and Ferreira (2006): head length, head depth, body depth, orbit diameter, caudal peduncle depth, caudal peduncle length, maximum body depth, and maximum body width. All individuals of *C. piquiti* were identified according to Kullander and Ferreira (2006), which morphologically described this species with four to five dark vertical bars on the lateral body and body colour varying from pale grey (more usual) to yellow. It presents a light ring around the caudal blotch which is always silvery to white (i.e. the eyespot), a lateral body with or without light spots, and anal and pelvic fins as long as the lower half of the caudal fin, which can range from yellowish to reddish in colouration. Male individuals (40–50 cm) present a reddish prominent nuchal protuberance. According to Kullander and Ferreira (2006), *C. piquiti* can be distinguished from other *Cichla* species by the presence of postorbital dark spots, absence of light spots on the anal fin, and absence of abdominal blotches.

#### *DNA isolation, amplification and sequencing*

Peacock cichlids were collected, dissected and kept frozen at  $-20^{\circ}\text{C}$  until DNA extraction. Total genomic DNA was extracted from 25 mg of muscle, using the NucleoSpin Tissue kit (Macherey-Nigel GmbH & Co. KG), following the manufacturer's instructions and

then stored at  $-20^{\circ}\text{C}$ . Two mitochondrial (mtDNA) molecular markers, control region (CR) and cytochrome *b* (*cytb*) were used to infer genetic differences between *Cichla* species in Lajes reservoir, through sequencing. The pair of primers tPro2-5 (5'-ACCC TAACTCCCAAAGC-3') and HN-20-3 (5'-GTGTT ATGCTTTAGTTAAGC-3') (Lee et al. 1995; Palumbi 1996) was used to amplify the CR loci. The *cytb* region was amplified via PCR with the primers GLUDG-5' (5'-CGAAGCTTGACTTGAARAACCA YCGTTG-3') and Cytb3-3' (3'-GCCAAATAGGAA RTATCATTC-5') (Lee et al. 1995; Palumbi 1996). For the amplification of both loci, the reaction mixture (25  $\mu\text{L}$ ) contained 1 unit (U) Taq DNA polymerase (ThermoScientific Inc., USA), 1 $\times$  reaction buffer with  $\text{NH}_4\text{SO}_4$ , 2.5 mM  $\text{MgCl}_2$ , 0.16 mM dNTPs (ThermoScientific Inc., USA) 8 pmol of each primer and 5 ng of genomic DNA. The PCR cycling for mitochondrial CR comprised the following thermocycling conditions: initial 5 minute heating step at  $94^{\circ}\text{C}$ , followed by 40 cycles of  $94^{\circ}\text{C}$  for 30 sec,  $45^{\circ}\text{C}$  for 30 s,  $72^{\circ}\text{C}$  for 1 min, and a final extension at  $72^{\circ}\text{C}$  for 5 min. The amplification for *cytb* were successful using the same thermocycling conditions that were used for CR loci, except for the extension step of the cycle which lasted 15 sec. PCR products from CR and *cytb* were purified and sequenced by Macrogen Inc. (Seoul, Korea). The accurate sequencing of the 3' portion of CR, downstream of a poly-T region, is problematic (Willis et al. 2007). Thus, to compensate for not having the reverse sequence,  $\sim 520$  bp of the 5' end were sequenced twice using the tPro2-5 forward primer and used in the analysis (Marques et al. 2016). Cytochrome *b* sequences were obtained using the same PCR primers described above. Sequences of *cytb* and CR were also obtained, using same procedure, for six individuals of *C. kelberi* to insure that this species of peacock cichlid introduced in Lajes reservoir for more than 60 years is not genetically the same as that obtained for our putative samples of *C. piquiti*.

#### *Data analysis*

Twenty-six sequences from *C. intermedia*, *C. jariina*, *C. kelberi*, *C. mirianae*, *C. monoculus*, *C. ocellaris*, *C. orinocensis*, *C. pinima*, *C. piquiti*, *C. temensis*, *C. thyrurus* and *C. vazzoleri* available on GenBank for the *cytb* region, along with nine sequences of *C. kelberi* and *C. piquiti* obtained in this study were used in the phylogenetic analysis. For the CR mtDNA loci, the same was done for the species quoted above plus *C. melaniae* and *C. pleiozona*, totalling 39 sequences, along with nine sequences obtained in this study. The *cytb* and CR mtDNA individual consensus

sequences were aligned using the MUSCLE algorithm separately. Therefore, one Maximum Likelihood (ML) phylogenetic tree with 1000 bootstrap replications was built for each molecular marker. The best-fit substitution models selected were HKY+G (*cytb*) and TN93+G (CR). A *Retroculus* sp. and a CR sequence of *Satanoperca* sp. were used as outgroups. In order to facilitate the comparison between different studies, the intraspecific and interspecific p-distance, based on *cytb* and CR sequences, were also determined. All cited procedures were performed with MEGA 6.06 software (Tamura et al. 2013).

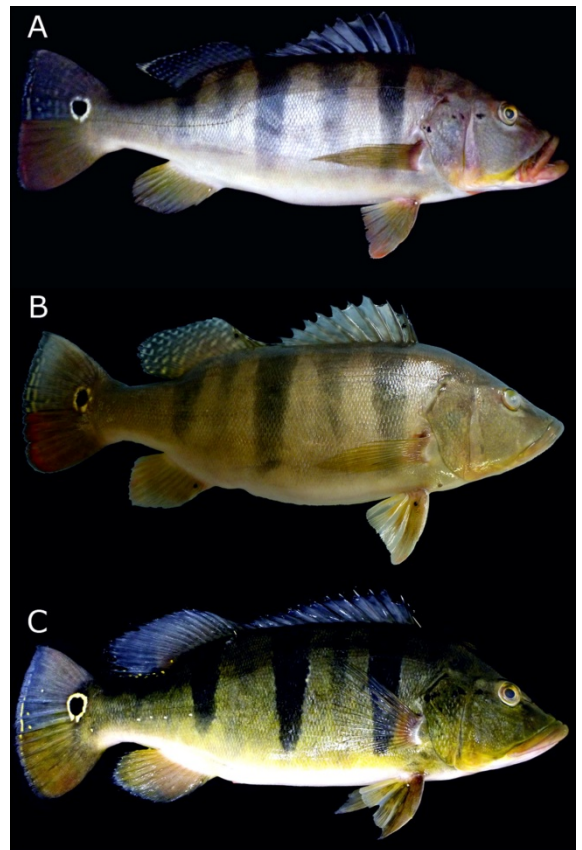
## Results

### *Morphological classification*

A total of 112 peacock cichlids were captured after 14 days (ca. 98 hours) of angling effort in Lajes reservoir, with three fish externally resembling *C. piquiti* (Figure 2). These three peacock cichlids showed five wide dark vertical bars below the dorsal fin (bars 1–3, 1a, 2a), with bars 1a and 2a paler than 1–3. Soft dorsal and anal fins were rounded, while pectoral fins were pointed, extending halfway to the origin of the soft anal fin. General body colour varied from pale grey to yellowish-green, with the lower half of the caudal fin being yellowish to reddish. Postorbital dark spots and small white spots were present on the soft dorsal fin as well as on the upper half of the caudal fin. Light spots were not recorded on pelvic and anal fins, and abdominal blotches were also lacking. Colouration patterns and morphological measurements of these three peacock cichlids revealed that, except for orbit diameter and head depth (Table 1), all of them matched the classification for *C. piquiti* available at Kullander and Ferreira (2006) and the holotype (HT–MZUSP 40296).

### *Genetic analysis*

The size of the PCR products obtained through 1.0% agarose gel were ~650 bp and ~550 bp for *cytb* and CR mtDNA regions, respectively. Multiple sequence alignment provided a matrix containing 655 and 532 sites for *cytb* and CR markers, respectively. Only one *cytb* and one CR haplotype were identified among the three *C. piquiti* specimens from the Lajes reservoir. A single haplotype, one for *cytb* and one for CR, was also found for the six *C. kelberi* analysed from Lajes reservoir. The Maximum Likelihood phylogenetic analysis based on the *cytb* and CR sequences revealed that the three *C. piquiti* specimens



**Figure 2.** Photographs of the three individuals of *Cichla piquiti* captured in Lajes reservoir, showing the variation in colour patterns: A–350 mm TL; B–402 mm TL; C–356 mm TL. Photo by Ana Clara Franco.

caught in Lajes reservoir clustered with other *C. piquiti* sequences available in the GenBank database, forming a well supported clade with bootstrap values of 99% for both markers (Figures 3 and 4), supporting its taxonomic assignment as such. The same occurred with our six *C. kelberi* specimens, as they also clustered with other *C. kelberi* sequences available in GenBank, with bootstrap values of 85% and 91% for *cytb* and CR, respectively (Figures 3 and 4). Intraspecific molecular variation was approximately 25 times lower than the genetic divergence among species for both markers. The p-distance for sequences within each *Cichla* species averaged 0.2% and 0.4% for *cytb* and CR loci, respectively, contrasting to 5.2% (*cytb*) and 10.7% (CR) among *Cichla* spp. sequences. The p-distance between sequences of *C. piquiti* and *C. kelberi* from the Paraíba do Sul river basin (i.e. this study) ranged from 6.2% (*cytb*) to 13.3% (CR), higher than the average values found for all *Cichla* sequences.

**Table 1.** Morphometric measurements of *C. piquiti* (mean; min-max) caught in Lajes reservoir and comparisons with those used for morphotaxonomical description (Kullander and Ferreira 2006) and from holotype (HT – MZUSP 40296). Measurements are in percent of SL, except SL, mouth depth, and mouth width (mm).

| Traits                     | Present study<br>(N=3) | Kullander and Ferreira (2006)<br>(N=20) | Holotype |
|----------------------------|------------------------|---|----------|
| SL (mm)                    | 325.8 (297.5 – 382)    | 192.6 (65.2 – 298.0)                    | 219.0    |
| Head length (%)            | 33.0 (30.5 – 34.9)     | 31.8 (30.6 – 35.0)                      | 31.4     |
| Head depth (%)             | 19.0 (18.1 – 19.7)     | 22.0 (20.9 – 23.6)                      | 22.6     |
| Body depth (%)             | 28.3 (25.7 – 29.8)     | 28.3 (26.0 – 30.7)                      | 30.3     |
| Orbit diameter (%)         | 5.0 (4.3 – 5.6)        | 7.9 (6.7 – 11.2)                        | 7.5      |
| Caudal peduncle depth (%)  | 11.1                   | 11.1 (10.0 – 12.1)                      | 12.1     |
| Caudal peduncle length (%) | 16.5 (15.0 – 17.8)     | 17.8 (16.0 – 19.9)                      | 17.7     |
| Caudal peduncle width (%)  | 7.7 (6.7 – 8.3)        |   |          |
| Maximum body width (%)     | 13.7 (13.0 – 15.0)     |   |          |
| Mouth depth (mm)           | 14.8 (14.4 – 15.6)     |   |          |
| Mouth width (mm)           | 17.3 (15.2 – 20.2)     |   |          |

## Discussion

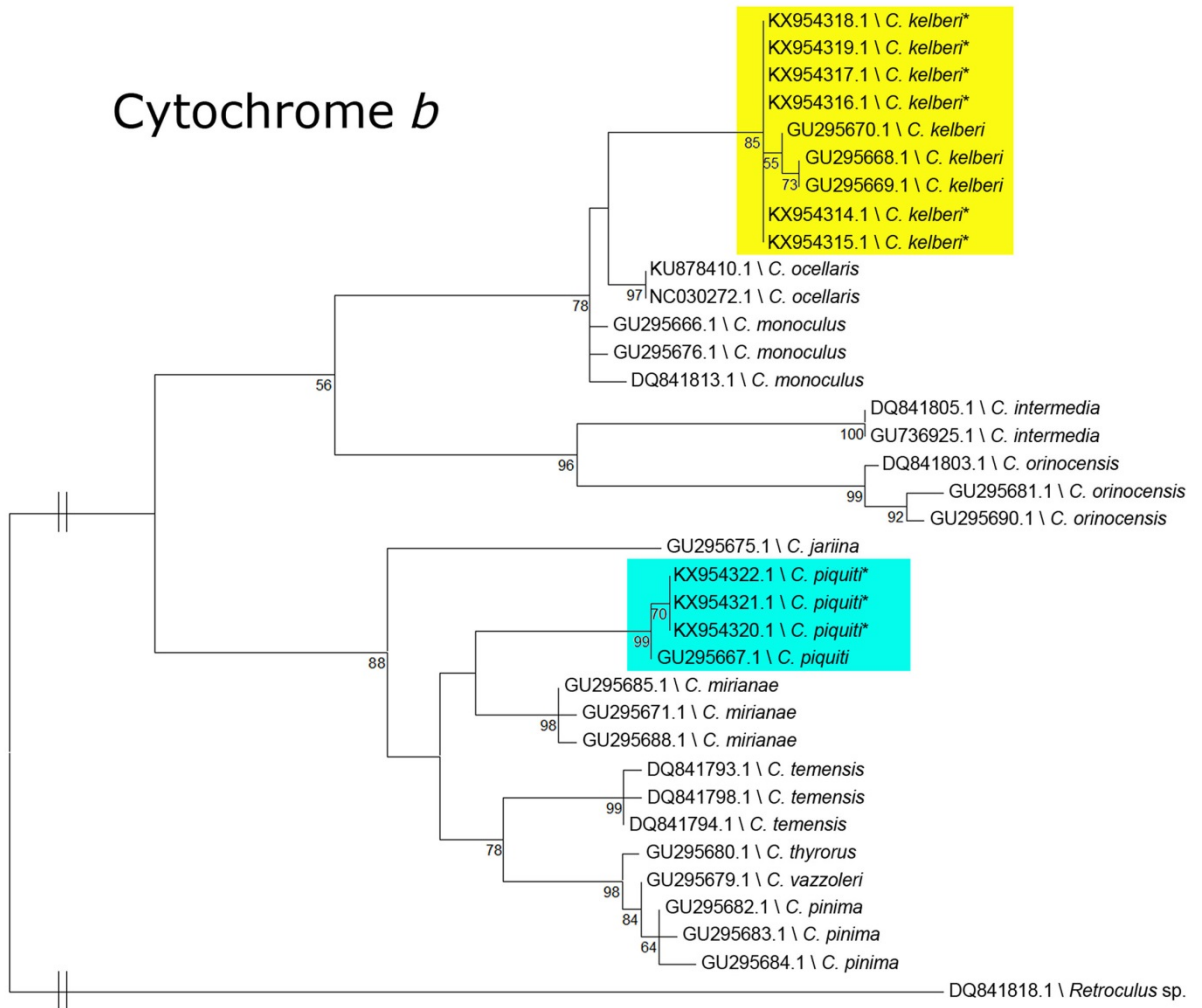
Morphological descriptors combined with genetic analyses based on two mitochondrial markers confirmed our hypothesis that the three peacock cichlids captured in Lajes reservoir are *C. piquiti*. Deviations were found only for orbit diameter and head depth, but these were probably related to the high size range of the *C. piquiti* captured in this study. Contrasting with other *Cichla* species, both morphotaxonomical (Kullander and Ferreira 2006) and molecular (Willis et al. 2012) studies agree in recognising *C. piquiti* as a valid species. Despite the considerable variation in body colour (i.e. from pale grey to yellow), our findings corroborated previous studies in that the blue peacock cichlid can be easily recognized and distinguished from other *Cichla* species by the presence of five dark vertical bars and dark postorbital marks.

Despite the ease with which *C. piquiti* may be recognised within its native distribution, this seems not to be the case for species detection and accurate identification in invaded systems, perhaps because of probable hybridisation with other species of peacock cichlids. *Cichla piquiti* is restricted to the Tocantins-Araguaia river basin, sharing the same native geographic distribution of *C. kelberi* and *C. pinima* (Kullander and Ferreira 2006). Hybrids of *C. piquiti* and *C. pinima* are considered rare in natural systems (Willis et al. 2012), but hybridisation between *C. piquiti* × *C. kelberi* is apparently common in invaded environments (Oliveira et al. 2006; Almeida-Ferreira et al. 2011). Although the hybrid *C. piquiti* × *C. kelberi*, if not already present in Lajes reservoir, is expected to occur, the use of mtDNA markers was very useful to confirm that the three individuals caught in this system have morphological and genetic features of *C. piquiti*.

*Cichla piquiti* was introduced into Lajes reservoir through deliberate releases by anglers, as reported by the affiliates of a local sportfishing club located near the dam. This pattern differed from the introduction of *C. kelberi* around the 1950s in the same environment (Santos et al. 2001), in which the yellow peacock cichlid was translocated directly from the Amazon region (i.e. native system) or small ponds in the north eastern semi-arid region (i.e. non-native system) through government agencies (Marques et al. 2016). The apparent failure of the first initiative carried out in 2005 to introduce *C. piquiti* in Lajes reservoir might be explained by the great vulnerability to predation and thus high mortality that the 1,000 fingerlings (~50mm) blue peacock cichlids probably experienced upon their arrival in the reservoir. This probable failure can be confirmed by the absence of published studies reporting *C. piquiti* in Lajes reservoir until 2008 (Yamada et al. 2011; Araújo and Rocha 2012), and the absence of this species in exploratory fisheries (LNS unpublished data) until 2010. The second attempt to introduce *C. piquiti* succeeded in 2010; the use of 400 young fish of greater size (200–250 mm TL) and thus less vulnerable to predation by adult *C. kelberi* was in turn crucial to assure greater survival and allow for the establishment of the blue peacock cichlid in Lajes reservoir. The existence of a long-term assessment programme for the peacock cichlid populations in Lajes reservoir, which started before 1995 (Araújo and Santos 2001; Santos et al. 2001), was thus of great importance in providing early recognition of the presence of *C. piquiti* just a few years after its successful introduction.

In addition to the first report of the introduction and establishment of *C. piquiti* in Lajes reservoir, a system with no records of *C. piquiti* until now, despite



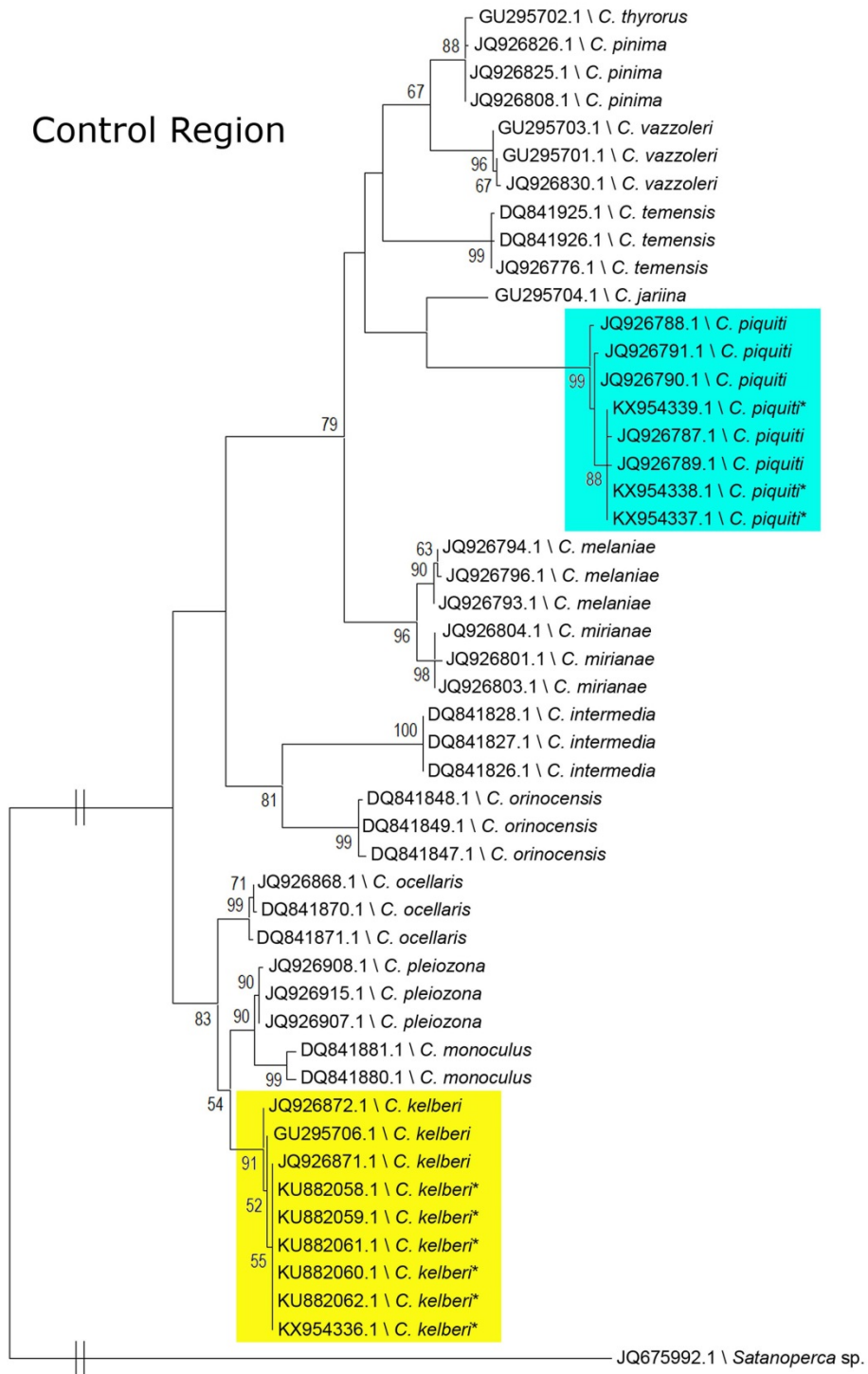


**Figure 3.** Maximum Likelihood tree based on *Cichla* spp. cytochrome *b* (cytb) sequences. For ease of comparison *C. piquiti* is highlighted in blue and *C. kelberi* is highlighted in yellow. Numbers at nodes represent bootstrap values after 1000 replications (only values above 50% are displayed). \*New sequences published in the present study.

the presence of a local sportfishing club and blue peacock cichlid populations in nearby systems (Oliveira et al. 2006; Carvalho et al. 2009; Almeida-Ferreira et al. 2011; Franceschini et al. 2013; Suárez et al. 2013; Santos et al. 2015), the present study also documents for the first time the presence of an invasive population of *C. piquiti* in the Paraíba do Sul river basin, one of the most important and largest rivers in south eastern Brazil. Surprisingly, there are no studies that have addressed the impacts of *C. piquiti* in the invaded systems or on native biota, even though detrimental effects on native fish, zooplankton, and phytoplankton assemblages are reported for other *Cichla* species (Zaret and Paine 1973; Latini and Petrere Jr. 2004; Menezes et al.

2012; Pelicice et al. 2015). However, since *C. piquiti* can apparently achieve higher maximum size (~700 mm TL) than *C. kelberi* (~600 mm TL), it is expected that the vulnerability of native fish to predation will be broader in the presence of invasive populations of *C. piquiti*.

The consequences of the establishment of *C. piquiti* for native fish assemblages in Lajes reservoir might be wrongly considered as of minor local importance, affecting mostly the management initiatives for recovering populations of native characids, such as the tiete tetra *Brycon insignis* Steindachner, 1877 and *Brycon opalinus* (Cuvier, 1819) (Santos et al. 2008). However, the impact of the introduction of *C. piquiti* has the potential to be much greater at larger



**Figure 4.** Maximum Likelihood tree based on *Cichla* spp. Control Region (CR) sequences. For ease of comparison *C. piquiti* is highlighted in blue and *C. kelberi* is highlighted in yellow. Numbers at nodes represent bootstrap values after 1000 replications (only values above 50% are displayed). \*New sequences published in the present study.

scales, when the geographic location of Lajes reservoir and the potential of *C. piquiti* to invade interconnected systems are taken into account. Lajes reservoir is directly connected to at least two reservoirs downstream (i.e. Pereira Passos and Paracambi), and also the Guandu river (Marques et al. 2016), the major source of drinking water for Rio de Janeiro State. In addition to the potential of passively reaching downstream systems through pumped waters from Lajes reservoir, *C. piquiti* can be actively transferred by local anglers not only to nearby reservoirs but also to other more distant systems in the Paraíba do Sul river basin, following a similar trend to that which apparently occurred for *C. kelberi* (Marques et al. 2016). Therefore, the combined use of morphotaxonomical and molecular approaches to assess peacock cichlid populations is key for early detection of the spread of these invasive fish within the Paraíba do Sul river basin and along to other nearby systems. These approaches could be also useful in the proper recognition of *Cichla* hybrids and to provide further information on the origin and number of introduction events of peacock cichlids.

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