REGULAR PAPER

Molecular and morphological diversity in species of *Kronichthys* (Teleostei, Loricariidae) from Atlantic coastal rivers of Brazil

Camila S. Souza[1] | Gabriel S. C. Silva[2] | Luz E. Ochoa[3] | Fabio F. Roxo[2] |
Guilherme J. Costa-Silva[4] | Fausto Foresti[1] | Bruno F. Melo[1] |
Claudio Oliveira[1]

[1]Departamento de Biologia Estrutural e Funcional, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil

[2]Departamento de Bioestatística, Biologia Vegetal, Parasitologia e Zoologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, Brazil

[3]Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil

[4]Universidade Santo Amaro, São Paulo, Brazil

Correspondence
Camila S. Souza, Departamento de Biologia Estrutural e Funcional, Instituto de Biociências, Universidade Estadual Paulista, R. Prof. Dr. Antônio C. W. Zanin 250, 18618-689 Botucatu SP, Brazil.
Email: camila.s.souza@unesp.br

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Abstract

The Neotropical catfish genus *Kronichthys* contains three species distributed along coastal rivers of southern and southeastern Brazil. Although phylogenetic hypotheses are available, the molecular and morphological diversity and species boundaries within the genus remain unexplored. In this study, the authors generated mitochondrial data for 90 specimens combined with morphometric and meristic data to investigate species diversity, species boundaries and putative morphological signatures in *Kronichthys*. Phylogenetic and species delimitation results clearly show the presence of four genetic lineages, three within *Kronichthys heylandi* along the coast from Rio de Janeiro to southern São Paulo and a single lineage encompassing both the nominal species *Kronichthys lacerta* and *Kronichthys subteres* from the Ribeira de Iguape basin to Santa Catarina in southern Brazil. Nonetheless, morphological data show overlapped ranges in morphometrics and a definition of only two morphotypes, with clear phenotypic differences in the teeth number: *K. heylandi* differs from *K. subteres* + *K. lacerta* by the higher number of premaxillary teeth (30–52 *vs.* 19–28) and higher number of dentary teeth (28–54 *vs.* 17–28). Headwater captures and connections of paleodrainages because of sea-level fluctuations represent the two major biogeographic processes promoting species diversification and lineage dispersal of *Kronichthys* in the Atlantic coastal range of Brazil.

KEYWORDS

diversity, Hypoptopomatinae, Serra do Mar, species delimitation, systematics

1 | INTRODUCTION

Neotropical armoured catfishes of the family Loricariidae comprise 1009 valid species (Fricke *et al.*, 2020) broadly distributed through most aquatic habitats and geographic regions of tropical South and Central America (Reis *et al.*, 2003). Species of the family can be easily recognized by having armoured bodies with ossified dermal plates and a ventrally positioned, disk-shaped mouth (Lujan & Armbruster, 2012). The family is currently divided into six subfamilies (Lujan *et al.*, 2015; Roxo *et al.*, 2019): Delturinae (7 species), Lithogeninae (3 species), Loricariinae (253 species), Hypoptopomatinae (249 species), Hypostominae (486 species) and Rhinelepinae (6 species) (Fricke *et al.*, 2020). Among the six large clades of Hypoptopomatinae (*sensu* Roxo *et al.*, 2019), the tribe Neoplecostomini contains middle-sized species inhabiting rock-bottom and fast-flowing

upland rivers of the Brazilian Shield included in *Euryochus* Pereira & Reis, 2017, *Hirtella* Pereira *et al*., 2014, *Isbrueckerichthys* Derijst, 1996, *Kronichthys* Miranda Ribeiro, 1908, *Neoplecostomus* Eigenmann & Eigenmann, 1888, *Pareiorhaphis* Miranda Ribeiro, 1918, *Pareiorhina* Gosline, 1947, *Plesioptopoma* Reis *et al*., 2012 and *Pseudotocinclus* Nichols, 1919 (Pereira & Reis, 2017; Roxo *et al*., 2019).

The monophyly of *Kronichthys* is supported by five exclusive synapomorphies, supplemented by 25 non-exclusive synapomorphies (Pereira & Reis, 2017), and supported by both multilocus and phylogenomic approaches (Roxo *et al*., 2012a, 2014, 2019). *Kronichthys* was described by Miranda Ribeiro to include *Kronichthys subteres* Miranda Ribeiro, 1908 (type species), based on four syntypes collected in the Betarí, Pardo and Iporanga rivers, all drainages of the Ribeira de Iguape basin (Miranda Ribeiro, 1908). The genus remained monotypic until the reallocation of *Plecostomus heylandi* Boulenger, 1900 into *Kronichthys* with the publication of the Loricariidae Catalogue (Isbrücker, 1980). Boulenger (1900) described *P. heylandi* (= *Kronichthys heylandi*) from a stream near Santos, southeastern Brazil, but it is now reported to occur also between Santos and Rio de Janeiro (RJ) (Weber, 2003). *Plecostomus lacerta* Nichols, 1919 was described from Poço Grande, Juquiá River, state of São Paulo (SP), Brazil (Nichols, 1919) and now is reported to occur between Paranaguá Bay and Ribeira de Iguape basin (Weber, 2003). The species was transferred to *Kronichthys* based on various morphological features (Armbruster & Page, 1997). Nonetheless, the authors pointed out the lack of morphological diagnoses to discriminate *Kronichthys lacerta* from the other two congeners because of insufficient details in the original description papers (see Armbruster & Page, 1997).

Recent molecular phylogenies based on multilocus and time-calibrated analyses, which included the three already-recognized species of *Kronichthys* and one undescribed taxon (*Kronichthys* sp.1), have hypothesized the interspecific relationships in *Kronichthys* and supported the monophyly of each species (Roxo *et al*., 2012a, 2012b, 2014). The results revealed that *Kronichthys* sp.1 from Mongaguá, southern coast of São Paulo, represented the earliest split from the remaining groups during the Late Miocene, and that *K. heylandi* diverged from the clade with *K. lacerta* and *K. subteres* more recently in the Early Pliocene (Roxo *et al*., 2014). The morphological phylogeny provided support for the same interspecific arrangement (*Kronichthys* sp. (*K. heylandi* (*K. lacerta* + *K. subteres*))) and additionally indicates *Kronichthys* sp. from the Macacu River, RJ, as a putative second undescribed species (Pereira & Reis, 2017). Despite the evidence of additional undescribed diversity, those studies included no more than five specimens of the genus and neither test species boundaries to elucidate taxonomic and systematic issues that persist nowadays within *Kronichthys*.

Species delimitation studies have challenged the number of recognized species in several otophysan groups (García-Melo *et al*., 2019; Machado *et al*., 2017, 2018; Mateussi *et al*., 2017, 2019; Melo *et al*., 2016a, 2018; Serrano *et al*., 2019; Silva *et al*., 2013). Subsequent taxonomic papers describing the hidden diversity exemplify a real progress (Guimarães *et al*., 2019; Mateussi *et al.,* 2018; Melo *et al*., 2016b; Melo & Oliveira, 2017; Silva *et al*., 2016) with several

addressing loricariids such as *Ancistrus* (Prizon *et al*., 2017), *Corymbophanes* (Lujan *et al*., 2020), *Hypostomus* (Anjos *et al*., 2020; de Queiroz *et al*., 2020), *Neoplecostomus* (Roxo *et al*., 2015), *Pareiorhaphis* (Fagundes *et al*., 2020), *Pseudolithoxus* (Collins *et al*., 2018), *Rineloricaria* (Costa-Silva *et al*., 2015) and *Schizolecis* (Souza *et al*., 2018). Those molecular studies along with time-calibrated phylogenies have been used as a framework for biogeographic assumptions for coastal and inland loricariid taxa across the Serra do Mar mountain range in the Atlantic coast of Brazil (Roxo *et al*., 2014, 2019). Headwater captures and connections on the exposed continental shelf during sea-level fluctuations represent two frequently reported hypotheses explaining species diversification in that region (Albert & Reis, 2011; Ribeiro, 2006; Roxo *et al*., 2014; Thomaz & Knowles, 2018) that are supported by phylogeographic studies with freshwater fishes (Angrizani & Malabarba, 2018; Camelier *et al*., 2018; Lima *et al*., 2017; Thomaz *et al*., 2015; Wendt *et al*., 2019). Because of species endemicity and restricted distribution to the drainages of the Brazilian Shield, *Kronichthys* represents another model to study biogeographic processes underlying species diversification in the Serra do Mar mountain range.

In this context, the present study uses a combined approach including both morphological and molecular data to evaluate the species diversity in *Kronichthys*, test species boundaries and investigate putative morphological signatures for lineages. In addition, the authors discuss both headwater capture and paleodrainage hypotheses and their implications for the biogeography of *Kronichthys* in the Atlantic coast of southeastern and southern Brazil (SBR).

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

All specimens were collected in accordance with Brazilian laws through SISBIO/MMA permit n. 3245, and procedures for collection, maintenance and analyses followed the international guidelines for animal experiments through CEEAA IBB/UNESP protocol n. 304. Voucher specimens were fixed in 95% ethanol or 10% formalin and transferred to 70% ethanol for permanent storage. Specimens were identified based on the available literature given their morphology and current known distribution (Miranda Ribeiro, 1908; Nichols, 1919; Armbruster & Page, 1997; Weber, 2003; Menezes *et al*., 2007; Pereira & Reis, 2017). Current definitions of *Kronichthys* species were rarely explored with examples of overlapped diagnoses such as caudal peduncle depth and length of dorsal-fin base (Menezes *et al*., 2007). In addition, the authors of this study analysed the photographs of the holotype of *P. heylandi* (BMNH 1899.12.18.1), holotype of *P. lacerta* (AMNH 715) and syntypes of *K. subteres* (MNRJ 655 (4)). After the identification of specimens, the vouchers and tissues were deposited in the collection of the Laboratório de Biologia e Genética de Peixes, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP, Brazil (LBP).

2.2 | Molecular phylogenetics and species delimitation analyses

A total of 90 specimens of *Kronichthys* were analysed: 9 specimens of *K. heylandi* from coastal rivers of southern RJ and 27 from northern São Paulo (NSP); 35 specimens of *K. lacerta* from the Ribeira de Iguape basin in SP and coastal rivers of Paraná to Santa Catarina (SBR); 7 specimens of *K. subteres* from the Ribeira de Iguape basin in SP (SBR); and 12 specimens of *K.* aff. *heylandi* from the coastal rivers of southern São Paulo (SSP) (Figure 1). The authors merged *K. lacerta* and *K. subteres* in only one abbreviation (SBR) in light of the phylogenetic results as detailed below. A single specimen of *Neoplecostomus microps* (Loricariidae: Hypoptopomatinae: Neoplecostomini) was used to root trees, based on previous molecular phylogenetic studies indicating both *Kronichthys* and *Neoplecostomus* as members of the tribe Neoplecostomini (Roxo *et al.*, 2012a, 2019). Because this study aims to investigate species diversity, more outgroup taxa were not included. Voucher data are summarized in Supporting Information Table S1.

DNA extraction followed Ivanova *et al.* (2006), and partial sequences of the *cytochrome c oxidase subunit I* (*COI*) gene were amplified by PCR with primers FishF1/R1 (Ward *et al.*, 2005). PCR amplifications were performed in a total volume of 12.5 μl that included 1.25 μl of 10× buffer, 0.25 μl of MgCl$_2$ (50 mM), 0.2 μl of dNTPs (2 mM), 0.5 μl of each primer (5 mM), 0.1 μl of PHT Taq DNA polymerase (Phoneutria, Belo Horizonte, MG, Brazil), 1.0 μl of genomic DNA (200 ng) and 8.7 μl of ddH$_2$O. The thermocycling profile consisted of an initial denaturation (5 min at 94°C), followed by 30 cycles of chain denaturation (40 s at 94°C), primer hybridization (30 s at 50–54°C) and nucleotide extension (1 min at 68°C), and a final extension (8 min at 68°C). All PCR products were checked on 1% agarose gels and then purified with ExoSap-IT (USB Corporation,

Cleveland, OH, USA) following the manufacturer's instructions. The purified PCR products were submitted to sequencing reactions using BigDye Terminator v 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Waltham, MA, USA) and purified again by ethanol precipitation. Products were loaded onto an ABI 3130 DNA Analyser automatic sequencer (Applied Biosystems, Waltham, MA, USA).

Raw sequences were assembled to consensus using Geneious v7.1.9 (Kearse *et al.*, 2012) and posteriorly aligned with Muscle (Edgar, 2004) under default parameters. To evaluate the occurrence of substitution saturation, the authors of this study estimated whether the index of substitution saturation (Iss) was significantly lower than Iss.cAsym (asymmetrical topology) or Iss.Sym (symmetrical topology) using the method of Xia *et al.* (2003) in DAMBE v5.3.38 (Xia, 2013). Nucleotide variation and substitution patterns were estimated in MEGA v6.0 (Tamura *et al.*, 2013). The best-fit model of nucleotide evolution was estimated in PartitionFinder (Lanfear *et al.*, 2012) (GTR+G). The authors also estimated the best-fit model in MEGA v6.0 (Tamura *et al.*, 2013) (K2P+G) for the distance analysis because MEGA v6.0 does not incorporate all the models estimated by PartitionFinder.

The overall mean distance (among all specimens), the intraspecific distances (among specimens of each species group) and interspecific distances (among species groups) were estimated using the Kimura-2-parameter (K2P) model (Kimura, 1980) in MEGA v6.0 (Tamura *et al.*, 2013). Groups were ordered based on preliminary neighbour-joining (NJ) topologies. The NJ tree was generated with 1000 bootstrap replicates in MEGA v6.0 (Tamura *et al.*, 2013). Maximum-likelihood (ML) analysis was conducted in RAxML PTHREADS-SSE3 implemented in RAxML v8.019 (Stamatakis, 2014) using the GTR+G model (Stamatakis *et al.*, 2008) as estimated by PartitionFinder. RAxML executed five searches with distinct randomized parsimony topologies, generated 1000 bootstrap replicates
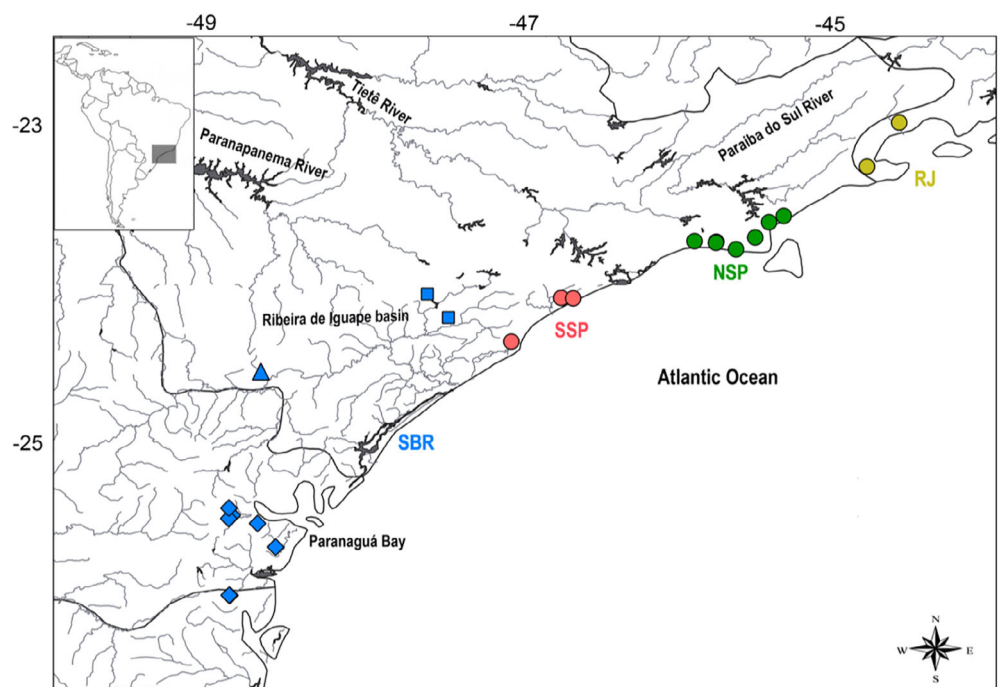

FIGURE 1 Map of southeastern Brazil showing the distribution of *Kronichthys* specimens included in molecular and morphological analyses. Distribution abbreviations: RJ: Rio de Janeiro; NSP: northern São Paulo; SSP: southern São Paulo; SBR: southern Brazil. *K. heylandi* ⬤; *K. heylandi* ⬤; *K.* aff. *heylandi* ⬤; *K. subteres* ▲; *K. lacerta* ◆; *K. lacerta* ◼

and applied the autoMRE function to test for convergence of the replicates (Pattengale *et al*., 2010). Species delimitation analyses included two approaches: (a) the Automatic Barcode Gap Discovery analysis (ABGD; Puillandre *et al*., 2012) available in the ABGD webserver (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html), excluding the root *Neoplecostomus microps* and under default parameters; and (b) Bayesian Poisson Tree Process (bPTP; Zhang *et al*., 2013) using the best ML tree, 100,000 generations and other parameters at default in the bPTP webserver (http://species.h-its.org/ptp/). In addition, the authors used DnaSP v5 (Librado & Rozas, 2009) with a reduced matrix excluding sites with missing data to estimate the haplotype diversity, haplotype number and haplotype distribution. Finally, a haplotype network was generated using the median-joining analysis (Bandelt *et al*., 1999) available in PopART 1.7 (Leigh & Bryant, 2015).

2.3 | Morphological analysis

Measurements and counts were taken from the left side of adult specimens (*i.e*., >48 mm standard length; SL) and were made point to point to the nearest 0.1 mm with digital callipers. Nomenclature for body plate follows Schaefer (1997) and for measurements and counts follows Carvalho and Reis (2009). Morphometric data are given as percentages of SL, except for subunits of the head region expressed as percentages of head length (HL). To account for the variation observed in the morphological data set of 65 specimens of *Kronichthys*, the data were transformed in log-ratio using the Aitchison transformation (Aitchison, 1986) using the function "AitTrans" from Pstat package in R (https://rdrr.io/cran/Pstat/man/). The transformed data set was used in the principal component analysis (PCA) (Jolliffe, 2002) using "prcomp" function of the stats package of the R software (R Development Core Team, 2011). The linear discriminant analysis (LDA) with the function "lda" was performed in the MASS package (Venables & Ripley, 2002). Specimens were assigned as follows: 10 specimens of *K. heylandi* (RJ), 15 specimens of *K. heylandi* (NSP), 10 specimens of *K. aff. heylandi* (SSP), 7 specimens of *K. subteres* (SBR) and 23 specimens of *K. lacerta* (SBR) (Supporting Information Table TABLE S2). Subsequently the cross-validation parameter was used as TRUE to perform cross-validation and provide posterior probabilities of group membership in the R software.

3 | RESULTS

3.1 | Molecular species delimitation

The final alignment comprised 91 terminals, 519 bp and 70 variable sites (13.5%). The reduced matrix for population genetic analyses included 480 bp and 66 variable sites (13.7%). Analyses of substitution saturation indicated no saturation in either transitions or transversions in both asymmetrical (Iss.cAsym) and symmetrical (Iss.cSym) topologies for both matrices. The overall mean of K2P genetic distances without outgroup was 0.027 ± 0.005. Intraspecific genetic variation ranged from 0.000 within *K*. aff. *heylandi* (SSP) and *K. heylandi* (RJ) to 0.006 ± 0.002 within the lineage *K. subteres* + *K. lacerta* (SBR). The values of interspecific distances among the four lineages ranged from 0.028 ± 0.008 between *K. heylandi* (RJ) and *K. heylandi* (NSP) to 0.042 ± 0.010 between *K*. aff. *heylandi* (SSP) and *K. heylandi* (RJ) (Table 1). The genetic distance between *K. subteres* and *K. lacerta* was only 0.011 ± 0.004.

The ML and NJ trees presented similar relationships and yielded four distinct lineages within *Kronichthys* (Figure 2a; Supporting Information Figure S1). The first lineage in the topology is composed of specimens of *K*. aff. *heylandi* from coastal rivers in Peruíbe-SP and Mongaguá-SP, SSP (100% bootstrap). The second includes specimens of *K. heylandi* from coastal rivers in Parati-RJ and Angra dos Reis-RJ, southern RJ (100% bootstrap). The third includes *K. heylandi* from coastal rivers in Caraguatatuba-SP, São Sebastião-SP and Bertioga-SP, NSP (95% bootstrap). The fourth lineage includes specimens of *K. subteres* from the Ribeira de Iguape basin in Iporanga-SP together with *K. lacerta* from Tapiraí-SP, Miracatu-SP, Paranaguá-PR, Morretes-PR, Matinhos-PR and Garuva-SC, coastal rivers from SSP, Paraná and northern Santa Catarina, Brazil (SBR; 97% bootstrap) (Figures 1 and 2).

Species delimitation results agree by the indication of four genetic lineages of *Kronichthys*, and most notably, revealed three lineages within the present concept of *K. heylandi* and only one encompassing two names, *K. lacerta* and *K. subteres* (Figure 2a). ABGD results (prior maximal distance *P* = 0.012915) indicated four lineages, splitting *K. heylandi* in three lineages and uniting *K. lacerta* and *K. subteres* in only one (Figure 2; Supporting Information Figure S2). The ML solution of the bPTP analysis returned identical results (Figure 2; Supporting Information Figure S3). Interestingly, the combined analyses revealed structured biogeographic patterns

TABLE 1 Pairwise K2P genetic distances within and among lineages of *Kronichthys*

	1	2	3	4
1. *Kronichthys* aff. *heylandi* SSP	**0.000 ± 0.000**			
2. *K. heylandi* RJ	0.042 ± 0.010	**0.000 ± 0.000**		
3. *K. heylandi* NSP	0.038 ± 0.009	0.028 ± 0.008	**0.002 ± 0.002**	
4. *Kronichthys subteres* + *Kronichthys lacerta* SBR	0.038 ± 0.009	0.038 ± 0.009	0.038 ± 0.008	**0.006 ± 0.002**

Note: Intraspecific genetic variation highlighted in bold. RJ: Rio de Janeiro; NSP: northern São Paulo; SBR: southern Brazil; SSP: southern São Paulo.
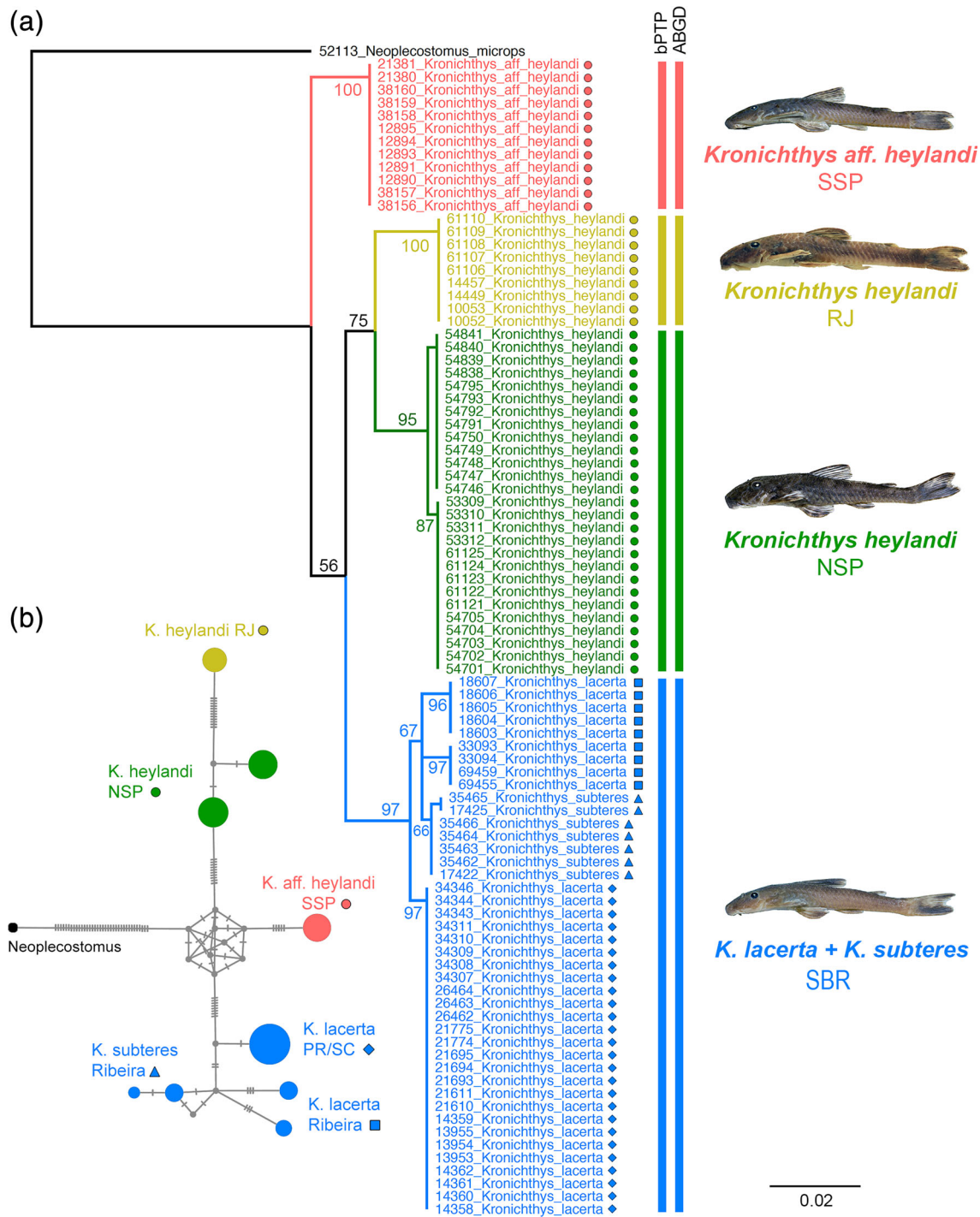
FIGURE 2 (a) Best maximum likelihood tree of *Kronichthys* based on partial sequences of the *cytochrome oxidase c subunit I*. Bars represent the number of species obtained by the bPTP and ABGD species delimitation analyses. Numbers near nodes represent bootstrap support, and those before tip names represent specimen numbers listed in Supporting Information Table S1. Colours and abbreviations follow Figure 1. (b) Haplotype network showing the distribution of the nine distinct haplotypes of *Kronichthys*. Distribution abbreviations: RJ: Rio de Janeiro; NSP: northern São Paulo; SSP: southern São Paulo; Ribeira: Ribeira de Iguape basin; PR/SC: coastal rivers of Paraná and Santa Catarina

in which the three lineages within the name *K. heylandi* appear distributed in distinct regions in the northernmost range (RJ, NSP and SSP) and the lineage of *K. lacerta* and *K. subteres* in the southernmost region (SBR) (Figures 1 and 2). Population genetic analyses resulted in a total of 10 haplotypes with haplotype diversity of

0.8479. The haplotype distribution was one haplotype to *Neoplecostomus* (root) and nine haplotypes for *Kronichthys*. *Kronichthys* aff. *heylandi* (SSP) and *K. heylandi* (RJ) presented one haplotype each, *K. heylandi* (NSP) and *K. subteres* (SBR) presented two haplotypes each and *K. lacerta* (SBR) presented three

	PC1	PC2	PC3	PC4	PC5
Standard length	−0.094	−0.003	−0.113	0.096	−0.151
Predorsal length	−0.028	−0.010	−0.087	0.054	−0.079
Preanal length	−0.008	−0.004	−0.118	0.094	−0.166
Head length	0.044	−0.005	0.056	0.070	−0.008
Cleithral width	0.160	−0.093	−0.004	−0.116	−0.055
Dorsal-fin spine length	−0.199	−0.197	0.178	0.206	0.142
Base of dorsal-fin length	−0.123	0.100	−0.067	−0.148	−0.191
Thorax length	−0.209	0.170	−0.296	0.026	−0.134
Pectoral-fin spine length	0.043	−0.351	0.005	−0.174	−0.154
Abdomen length	0.113	−0.070	−0.271	0.069	−0.320
Pelvic-fin spine length	−0.181	−0.146	0.221	0.064	0.021
Anal-fin spine length	−0.169	−0.133	0.272	0.135	0.136
Lower cd spine	−0.075	−0.343	0.146	0.331	0.179
Caudal peduncle depth	−0.140	0.560	0.537	−0.192	0.036
Caudal peduncle length	−0.236	−0.040	−0.224	0.112	−0.082
Anal width	−0.234	0.218	−0.067	−0.235	−0.277
Snout-opercle length	0.069	0.010	0.234	0.105	0.009
Head width	0.167	−0.046	−0.017	−0.109	0.048
Head depth	−0.023	0.115	0.058	−0.208	0.061
Snout length	0.163	0.121	−0.019	0.273	0.142
Interorbital width	−0.019	−0.012	−0.089	−0.059	0.071
Orbital diameter	0.049	−0.270	−0.145	−0.648	0.471
Suborbital depth	0.182	0.403	−0.388	0.244	0.542
Mandibular ramus length	0.747	0.028	0.201	0.009	−0.240

TABLE 2 Factor loadings of the first five principal components (PC1–PC5)

haplotypes (Figure 2b). Analyses indicated the lack of haplotype sharing among the four lineages.

3.2 | Morphological analysis

The PCA detected five PC axes, in which the first two axes together account for 60.1% of the body shape variation; the first axis explains 34.34%, and the second axis explains 25.76% of the variation in body shape for all the analysed *Kronichthys* specimens. Eigenvalues and percentage of explained variability are shown in Table 2, and the morphospace plotted on the first two axes and variables associated are shown in Figure 3a. According to the PC1 eigenvalues, the morphological characters with the greatest variations are mandibular ramus length (0.747), suborbital depth (0.182), head width (0.167), snout length (0.163) and cleithral width (0.160) (Table 2). PCA results indicated partial overlaps between *K. subteres* and *K. lacerta* (both SBR), and between *K. heylandi* (RJ), *K. heylandi* (NSP) and *K. aff. heylandi* (SSP) (Figure 3a). The LDA analysis was effective in discriminating the four genetic lineages of *Kronichthys* (Figure 3b) with only a slight overlap between *K. lacerta* and *K. subteres*. The cross-validation and posterior probabilities of molecular prior assignments indicated that most individuals are assigned with high probability to their specific lineages (Figure 4). A few events of clustering appear between *K.*

aff. *heylandi* (SSP) and *K. heylandi* (NSP) with posterior probabilities 0.001–0.038, and between *K. lacerta* and *K. subteres* (SBR) with posterior probabilities 0.003–0.999 (Figure 4).

Although the authors could not identify synapomorphies to support each of the four genetic lineages, they found additional characters that distinguish two morphotypes for the three currently recognized species. *Kronichthys heylandi lato sensu* [including *K. aff. heylandi* (SSP), *K. heylandi* (RJ) and *K. heylandi* (NSP)] can be differentiated from *K. subteres* + *K. lacerta* by the higher number of premaxillary teeth (31–52, 30–40 and 31–52 *vs.* 20–26 and 19–28, respectively) and higher number of dentary teeth (32–46, 28–36 and 30–54 *vs.* 17–28 and 18–26, respectively) (Table 3). Nonetheless, the authors did not find sufficient characters to distinguish either *K. heylandi* (RJ) or *K. aff. heylandi* (SSP), considering the fact that the lineage geographically closest to the type locality is presumably that from the NSP. In addition, analyses did not find morphological evidence to distinguish *K. lacerta* from *K. subteres*.

4 | DISCUSSION

Mitochondrial data and species delimitation analyses support the occurrence of four genetic lineages within *Kronichthys*, three of which in the present concept of *K. heylandi* distributed along coastal
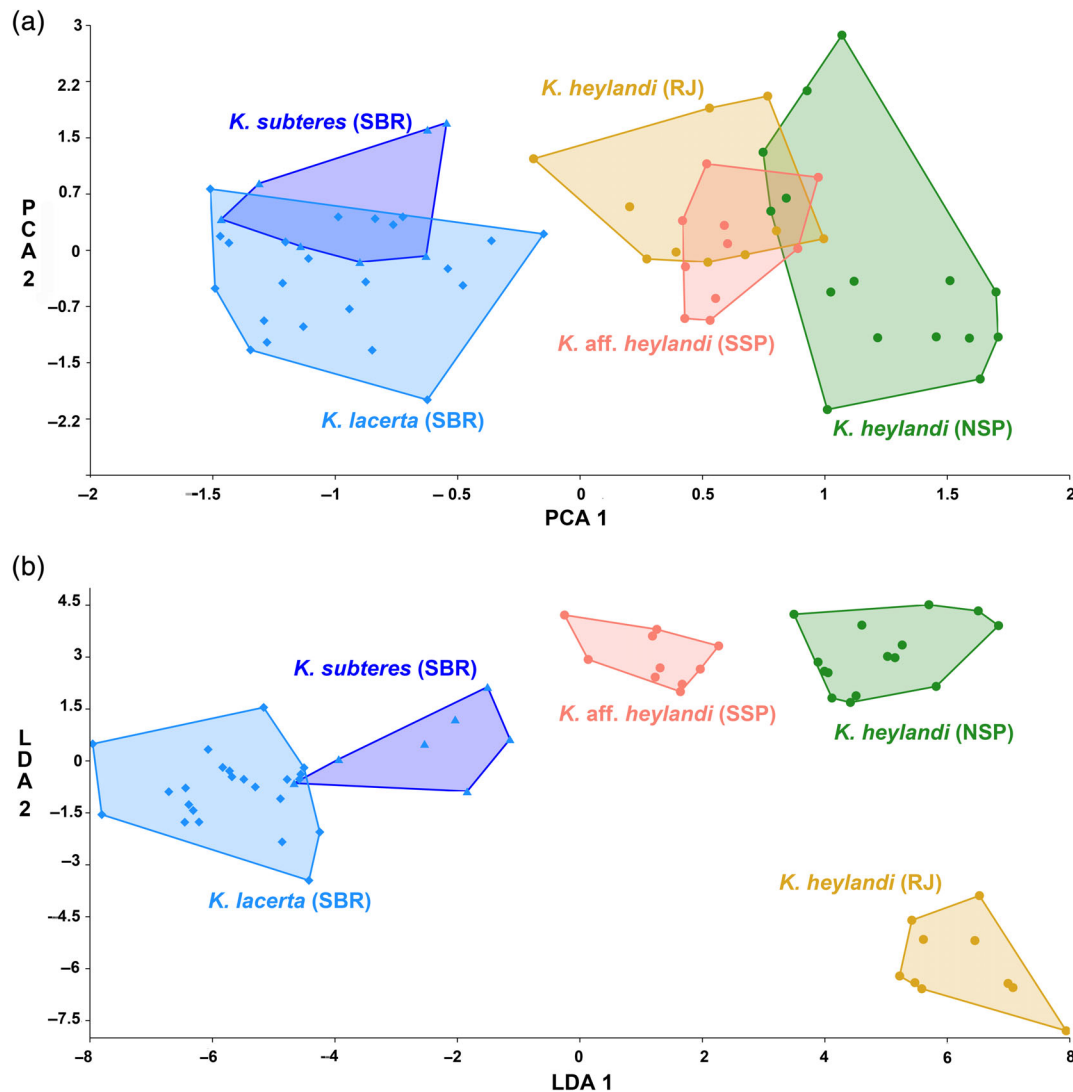
FIGURE 3 (a) Scatter plot of principal component analysis (PCA) of five groups of *Kronichthys* showing the morphometric overlaps. (b) Scatter plot of linear discriminant analysis (LDA) of five groups of *Kronichthys* showing a slight overlap between *K. subteres* and *K. lacerta*. Distribution abbreviations: RJ: Rio de Janeiro; NSP: northern São Paulo; SSP: southern São Paulo; SBR: southern Brazil

Atlantic rivers of Rio de Janeiro and São Paulo, and one lineage represented by *K. subteres* and *K. lacerta* from the Ribeira de Iguape basin and coastal rivers of Paraná and Santa Catarina (Figures 1 and 2). The arrangement with *K. aff. heylandi* sister to the clade with *K. heylandi* and then *K. lacerta* + *K. subteres* matches previous phylogenies using multilocus data (Roxo *et al.*, 2012a; Roxo *et al.*, 2012b; Roxo *et al.*, 2014) and, in part, by morphology (Pereira & Reis, 2017). Although both studies explicitly indicate the presence of undescribed diversity, the specimens of *K. aff. heylandi* analysed here from coastal rivers of SSP are the same species in the molecular study (Roxo *et al.*, 2012a; Roxo *et al.*, 2012b; Roxo *et al.*, 2014) but presumably not the species from the Macacu River, eastern Guanabara Bay in RJ (Pereira & Reis, 2017). The distinction is because of the current geographic distribution of the genetic lineages indicating a clear structure of lineages/species along the Brazilian coast, but certainly more studies should clarify this question.

The three proposed lineages within *K. heylandi* are morphologically indistinguishable from each other and present morphometric overlaps (Figure 3a). Because lineages of *K. heylandi* are indistinguishable morphologically, the molecular evidence showing three well-defined genetic lineages represents another example of cryptic diversity for Neotropical freshwater fishes (Guimarães *et al.*, 2019; Mateussi *et al.*, 2019; Melo *et al.*, 2016b). On the contrary, results consistently indicate a single genetic lineage for the nominal species *K. subteres* and *K. lacerta* (Figure 2). Both species were described from the Ribeira de Iguape basin: *K. subteres* from Betari, Pardo and Iporanga rivers (Miranda Ribeiro, 1908) and *K. lacerta* from Poço Grande, Juquiá River, SP (Nichols, 1919). PCA analyses revealed clear overlaps between the two species, and LDA showed only a slight overlap (Figure 3), although limited taxon sampling was available for *K. subteres*. Nonetheless, the present evidence based on both molecular and morphological data is not sufficient to support the nomenclatural changes at this moment.
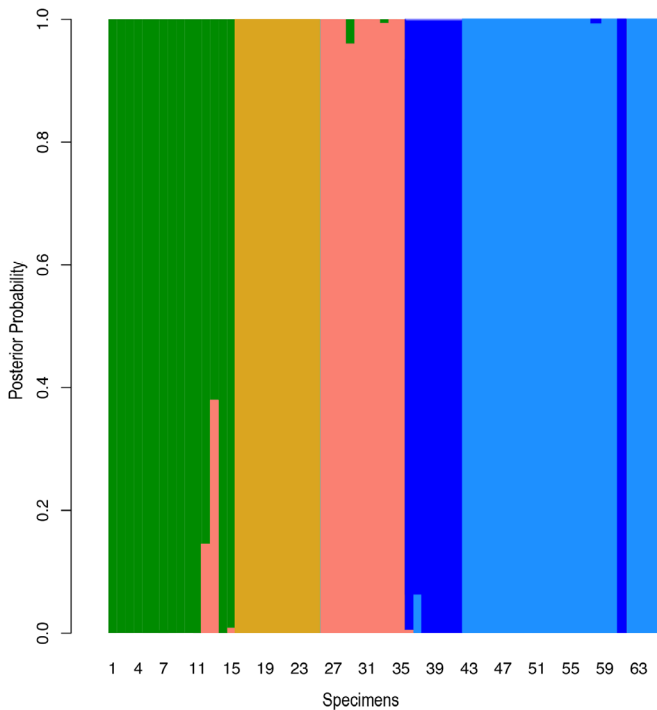
FIGURE 4 Cross-validation analysis for molecular lineages identified with posterior probabilities of individuals assigned to each group. Distribution abbreviations: RJ: Rio de Janeiro; NSP: northern São Paulo; SSP: southern São Paulo; SBR: southern Brazil

K. heylandi (NSP)
K. heylandi (RJ)
K. aff. *heylandi* (SSP)
K. subteres (SBR)
K. lacerta (SBR)

Further analyses incorporating more samples of *K. subteres*, possibly using genomic data, along with direct examination of types of both *K. subteres* and *K. lacerta* will surely provide stronger support for recognition of either one or two species in this clade.

Difficulties to delimit species of *Kronichthys* are mainly because of relatively old descriptions (Boulenger, 1900; Miranda Ribeiro, 1908; Nichols, 1919) that do not contain sufficient details of the diagnoses among species (Armbruster & Page, 1997). That is unsurprising given the fact that both Boulenger (1900) and Nichols (1919) described *K. heylandi* and *K. lacerta* as species of *Plecostomus* Gronow, a genus that once included many loricariids and is now under the synonym of the species-rich genus *Hypostomus* Lacepède. The LDA analysis in this study effectively discriminated the four molecular lineages (Figure 3b), but the authors did not find apomorphic characters to distinguish them. Nonetheless, the authors provide evidence for characters related to number of both premaxillary and dentary teeth that effectively distinguish the two species of *Kronichthys*: *K. heylandi* has a numerous premaxillary and dentary teeth, whereas the morphotype *K. subteres* has less premaxillary and dentary teeth (see Results). These traits are functionally relevant to the distinctive feeding habits of loricariids and are directly associated with dietary specializations (Lujan & Armbruster, 2012), an indicative that they occupy different trophic niches along the wide range of the southeastern Brazil coast.

Two main biogeographic processes explain events of geodispersal along the coastal rivers of southeastern Brazil: (a) headwater capture, a geomorphological process by which the river flow is diverted into the neighbouring basin (Bishop, 1995; Ribeiro, 2006; Roxo et al., 2014), and (b) Pleistocene paleodrainages, connections among coastal river systems during periods of marine regression (Dias

et al., 2014; Thomaz et al., 2015; Thomaz & Knowles, 2018). Faunal interchanges because of headwater captures in southeastern Brazil have often been reported for several clades of Neotropical freshwater fishes (Camelier et al., 2018; Ribeiro, 2006) including loricariids (Lima et al., 2017; Roxo et al., 2014). The phylogeographic pattern observed in the loricariid *Pareiorhaphis garbei* is explained by headwater captures between the São João basin and the rivers draining the Guanabara Bay in RJ (Lima et al., 2017). Evidence for river capture affecting speciation events has also been reported recently for species of *Characidium* in the Paraíba do Sul River basin (Pucci et al., 2014; Serrano et al., 2019). The split of *K.* aff. *heylandi* with other species is estimated to have occurred in the Late Miocene and the split of *K. heylandi* with the clade *K. lacerta* + *K. subteres* in the Early Pliocene (Roxo et al., 2014). At this period, several events of river capture have been hypothesized for the diversification of loricariids in the southeastern Brazilian Shield (Ribeiro, 2006; Roxo et al., 2012a; Roxo et al., 2012b; Roxo et al., 2014), which might have influenced the earlier split of *K.* aff. *heylandi* relative to other lineages of *Kronichthys*.

The other two clades appear to have had a range expansion along the southeastern and SBR coast (Figures 1 and 2). Pleistocene connections of paleodrainages because of marine regressions have explained the distribution pattern of genetic lineages of freshwater fishes in coastal rivers of southeastern Brazil. Previous studies have shown that dispersal events through past connection of paleodrainages could explain the intraspecific and interspecific genetic variation and evolutionary history of *Hollandichthys* (Thomaz et al., 2015), *Cyanocharax* (Hirschmann et al., 2015) and *Oligosarcus* (Wendt et al., 2019). These recent processes may have promoted lineage expansion throughout the both clades, with *K. heylandi* colonizing the northern coast of SP and RJ, and *K. lacerta* and *K. subteres* from the Ribeira de Iguape basin to coastal

TABLE 3 Morphometric data of *Kronichthys* specimens.

	Kronichthys aff. *heylandi* SSP (n = 10)		*K. heylandi* RJ (n = 10)		*K. heylandi* NSP (n = 15)		*Kronichthys subteres* SBR (n = 7)		*Kronichthys lacerta* SBR (n = 23)	
Standard length (SL; mm)	Min–max	Mean ± SD	Min–max	Mean ± SD	Min–max	Mean ± SD	Min–max	Mean ± SD	Min–max	Mean ± SD
	48.8–86.2	66.4 ± 12.8	60.2–84	68.6 ± 8.3	48.5–83.2	59.8 ± 9.9	48.6–81.9	61.1 ± 12.4	52.4–85.8	68.4 ± 8.4
Percentage of SL										
Predorsal length	42.8–45.6	44 ± 0.9	42.3–45	43.7 ± 0.8	42.8–47.2	44.6 ± 1.2	41.6–47	43.5 ± 1.8	40.4–45.2	42.9 ± 1.1
Preanal length	58.3–64.1	61.6 ± 1.6	58.3–62.7	60.2 ± 1.7	59.7–64.3	61.4 ± 1.4	57.6–60.6	59.3 ± 1.1	56.7–61.8	58.8 ± 1.3
Head length	29.6–35.9	31.4 ± 1.7	26.3–31.8	29.6 ± 1.9	30.2–33.2	31.6 ± 1	27.8–31.4	30 ± 1.4	27.7–31.9	29.3 ± 1.1
Cleithral width	22.1–25.3	23.9 ± 0.9	22.0–24.4	23.3 ± 0.7	23.1–27.1	25.2 ± 1.1	20.3–25.3	22.7 ± 1.7	19.6–24.3	21.6 ± 1.2
Dorsal-fin spine length	20.2–23.8	21.9 ± 1.3	19.1–23.2	20.5 ± 1.5	15.6–24.7	21.4 ± 2.2	19.2–24.2	22.4 ± 1.9	20.6–25	22.5 ± 1.3
Base of dorsal-fin length	13.9–16.3	15 ± 0.8	14.2–16.9	15.5 ± 0.8	14.4–17	15.8 ± 0.9	15.9–17.6	16.5 ± 0.6	14–17.8	15.4 ± 0.9
Thorax length	14.2–17.3	15.5 ± 0.8	15.2–18.1	16.8 ± 1.1	13.7–16.1	15.3 ± 0.8	15.9–18.6	17.2 ± 0.9	13.9–19.1	16.2 ± 1.2
Pectoral-fin spine length	17.8–22.5	21 ± 1.4	16.3–20.5	18.9 ± 1.5	17.5–24.1	21.1 ± 2.3	17.2–23.6	20.6 ± 2.4	16.8–22.9	19.2 ± 1.6
Abdomen length	23.5–26.7	25.4 ± 1.1	24.3–27.5	25.6 ± 1.1	23–26.6	25.1 ± 0.1	20.4–24.4	22.6 ± 1.3	21.2–26.0	22.9 ± 1.1
Pelvic-fin spine length	16.3–20.1	17.6 ± 1.3	15–17.7	16.3 ± 0.9	16.4–20.8	18.0 ± 1.1	17–19.7	18.2 ± 1	15.9–21.2	18.4 ± 1.1
Anal-fin spine length	13.3–16.3	15 ± 0.8	12.8–17.6	14.4 ± 1.6	13.4–16.3	15.4 ± 0.7	11.3–17.6	15.4 ± 2.2	14.2–16.8	15.6 ± 0.7
Lower caudal-fin spine length	21.2–26.7	23.4 ± 1.6	17–23.8	21.1 ± 2.2	18–26.4	23.4 ± 2.1	18.2–25.4	21.9 ± 2.8	20.5–27.5	23.3 ± 1.7
Caudal peduncle depth	7.6–11	9.1 ± 1.1	7.3–10	8.4 ± 0.9	7.3–17.3	9.1 ± 2.5	9.1–11.1	10.3 ± 0.7	7.2–10.3	8.5 ± 0.8
Caudal peduncle length	32–34.3	33.3 ± 0.8	31.4–41.5	35 ± 2.8	30.9–33.9	32.6 ± 0.8	33.2–38	34.7 ± 1.6	32.4–37.5	35.4 ± 1.6
Anal width	23.5–26.5	24.9 ± 0.9	14.5–17.1	15.9 ± 0.9	13.2–17.3	15.4 ± 1.6	16.9–19.5	18.4 ± 0.8	14.5–17.4	16 ± 0.8
Snout-opercle length	12.1–22.2	16.6 ± 3.2	20.4–24	22.1 ± 1.2	23.9–27	25.9 ± 0.8	22.5–24.9	23.6 ± 0.9	21.3–25.0	23.1 ± 1
Percentages of head length										
Head width	70.2–79.2	76.1 ± 2.7	74.6–91.7	79.3 ± 5.1	77.3–82.8	79.6 ± 2.1	71.6–80.4	75.8 ± 3.5	69.7–78	73 ± 2.2
Head depth	42.5–46.4	44.5 ± 1.1	42–52	46.5 ± 2.7	41.7–48.8	45.4 ± 1.9	49.2–51.9	50.6 ± 0.9	43–50.7	45.2 ± 2.1
Snout length	55.3–59.4	57.6 ± 1.2	55.7–69	62.3 ± 3.3	55.4–63.3	59 ± 2.2	53.5–58	55.3 ± 1.6	49.9–60.3	55.8 ± 2.9
Interorbital width	32.5–37.9	35.7 ± 1.6	38.7–46.3	41.4 ± 2.6	36.5–40.5	38.2 ± 1.1	36.2–41.5	39.4 ± 2.0	34.5–42.2	39 ± 1.7
Orbital diameter	10.1–13.7	12 ± 1.3	10.4–15.1	13.4 ± 1.4	10.9–16.4	13.4 ± 1.5	12.9–14.9	13.9 ± 0.7	10.4–15.2	12.7 ± 1
Suborbital depth	20.4–23.5	22.1 ± 1.0	22.4–30.7	26.7 ± 2.4	13.9–25.6	22.0 ± 2.8	21.2–24.8	22.8 ± 1.4	16.8–23.7	20.7 ± 1.8
Mandibular ramus length	15.5–18.7	17 ± 1.2	13.9–19.4	17 ± 1.6	17.3–21.3	19.6 ± 1.3	12.3–14.7	13.5 ± 0.9	11.7–15	13.4 ± 0.9
Meristics	Min–max	Mode	Min–max	Mode	Min–max	Mode	Min–max	Mode	Min–max	Mode
Medial plates	27–28	28	27–28	28	27–28	27	26–28	27	27–29	28
Premaxillary teeth	31–52	36	31–52	30	31–52	35	20–26	23	19–28	24
Dentary teeth	32–46	32	30–54	32	30–54	38	17–28	22	18–26	21

Note: RJ: Rio de Janeiro; NSP: northern São Paulo; SBR: southern Brazil; SD: standard deviation; SSP: southern São Paulo.

basins of Paraná and Santa Catarina. This is also supported by time-calibrated phylogenies indicating a more recent (Early Pleistocene) divergence between *K. lacerta* and *K. subteres* (Roxo *et al.*, 2014).

Overall, results from this study indicate the presence of four genetic lineages of *Kronichthys* and only two morphotypes that are effectively diagnosed. This contribution will help further investigations about the undescribed species within the present concept of *K. heylandi*, and the taxonomic status of *K. subteres* relative to *K. lacerta*. The authors suggest an extensive taxonomic revision of the genus including direct examination of types of both *K. subteres* and *K. lacerta* in combination with these genetic data. The authors also hypothesize that both river capture and Pleistocene interconnections of paleodrainages may have played an important role in the lineage dispersal and colonization of *Kronichthys* along southeastern and southern coast of Brazil.

AUTHOR CONTRIBUTIONS

C.S.S., B.F.M., G.S.C.S. and G.J.C.S. designed the ideas of the research. C.S.S. and G.S.C.S. collected the data. C.S.S., B.F.M. and L.E.O. performed the analyses. C.C.S. and B.F.M. wrote most of the text. G.S.C.S., L.E.O., F.F.R., G.J.C.S. and C.O. contributed with writing. F.F. and C.O. provided structure for the research.

ORCID

Camila S. Souza https://orcid.org/0000-0002-3878-2373
Gabriel S. C. Silva https://orcid.org/0000-0002-9843-3175
Luz E. Ochoa https://orcid.org/0000-0003-4205-8510
Fabio F. Roxo https://orcid.org/0000-0002-3982-4934
Guilherme J. Costa-Silva https://orcid.org/0000-0001-6380-8697
Fausto Foresti https://orcid.org/0000-0002-0862-0445
Bruno F. Melo https://orcid.org/0000-0002-0499-567X
Claudio Oliveira https://orcid.org/0000-0002-4143-7212

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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