



BARCODING NEOTROPICAL FISHES

Claudio Oliveira

Departamento de Morfologia
Instituto de Biociências - UNESP
Botucatu, São Paulo, Brazil
claudio@ibb.unesp.br



Neotropical Region



Fishes

> 50% of vertebrates are fishes = 28,000 species



From these 13,000 are freshwater species



**About 6,000 species are Neotropical
4,475 are valid species + 1,550 are undescribed**

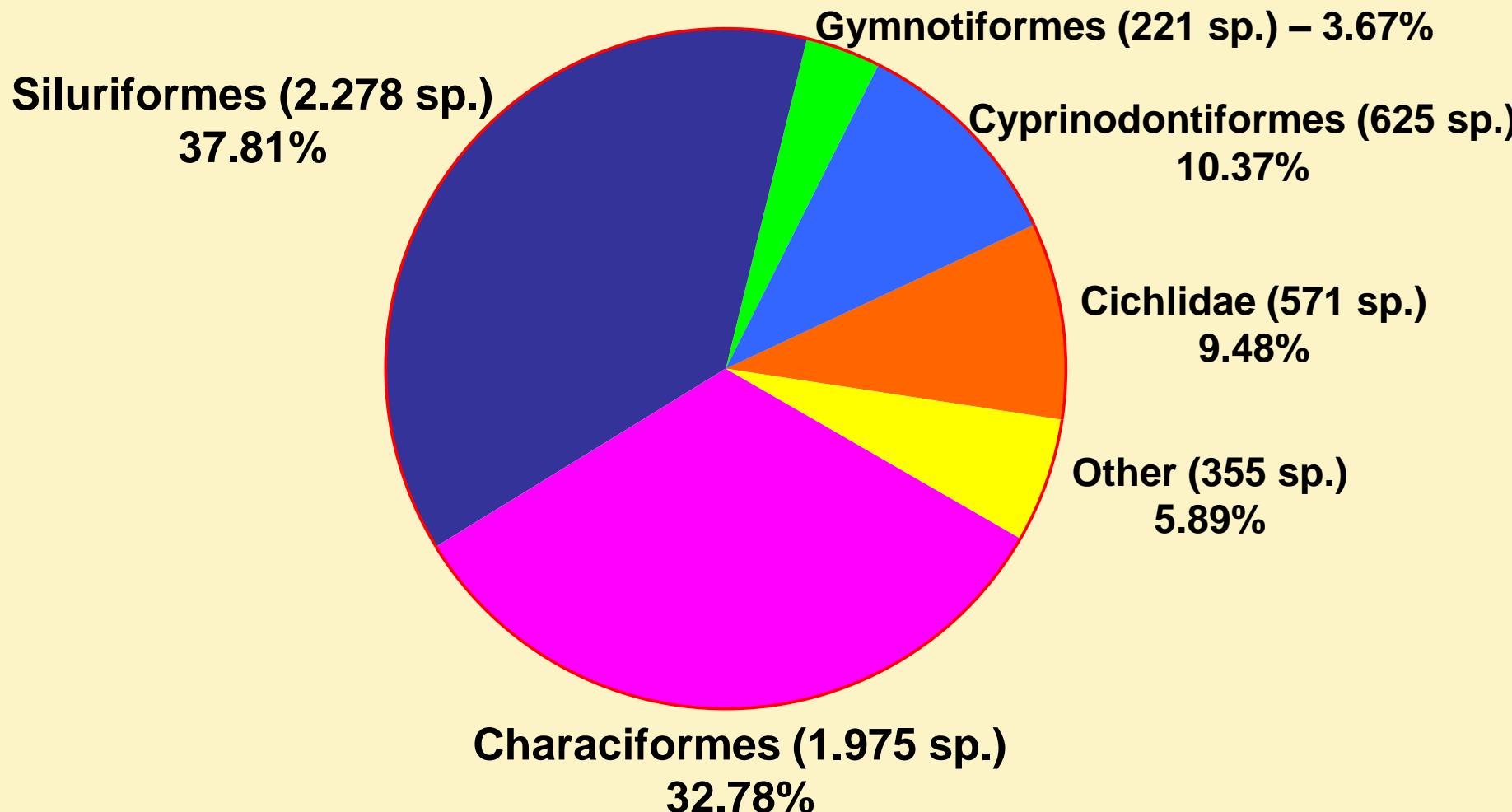


The richest freshwater fish fauna of the world

Neotropical freshwater fishes

4.475 named species (6.025 estimated)

(Reis et al., 2003)



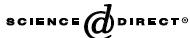
Brazilian saltwater fishes

	N	
Classe Myxini		Osmeriformes 19
Myxiniformes	4	Stomiiformes 58
Classe Condrichthyes		Aulopiformes 51
Chimaeriformes	3	Myctophiformes 80
Orectoboliformes	2	Lampridiformes 8
Carcharhiniformes	41	Polymixiiformes 2
Lamniformes	13	Ophidiiformes 28
Hexanchiformes	3	Gadiformes 33
Squaliformes	18	Batrachoidiformes 13
Squatiniformes	4	Lophiiformes 17
Rajiformes	55	Mugiliformes 7
Classe Actinopterygii		Atheriniformes 14
Elopiformes	2	Beloniformes 34
Albuniformes	4	Stephanoberyciformes 1
Anguilliformes	94	Beryciformes 14
Saccopharhyngiformes	1	Zeiformes 5
Clupeiformes	47	Gasterosteiformes 18
Siluriformes	21	Scorpaeniformes 32
		Perciformes 456
		Pleuronectiformes 56
		Tetraodontiformes 40
		Total 1297

Fish phylogeny



Available online at www.sciencedirect.com



ELSEVIER

Molecular Phylogenetics and Evolution 32 (2004) 152–163

MOLECULAR
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www.elsevier.com/locate/ympev

Molecular phylogeny of the armored catfish family Callichthyidae (Ostariophysi, Siluriformes)

Cristiane Kioko Shimabukuro-Dias,^a Claudio Oliveira,^{a,*} Roberto E. Reis,^b
and Fausto Foresti^a



Genetics and Molecular Biology, 31, 1 (suppl), 284–292 (2008)
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www.sbg.org.br

Research Article

Phylogenetic analysis of the order Pleuronectiformes (Teleostei) based on sequences of 12S and 16S mitochondrial genes

Marisa F.C. Azevedo^{1*}, Claudio Oliveira¹, Belén G. Pardo², Paulino Martínez² and Fausto Foresti¹

Copeia, 2004(3), pp. 496–506

Phylogenetic Analysis and Redescription of the Genus *Henochilus* (Characiformes: Characidae)

RICARDO M. C. CASTRO, RICHARD P. VARI, FÁBIO VIEIRA, AND CLAUDIO OLIVEIRA



ELSEVIER

Molecular Phylogenetics and Evolution 49 (2008) 606–617

Contents lists available at ScienceDirect

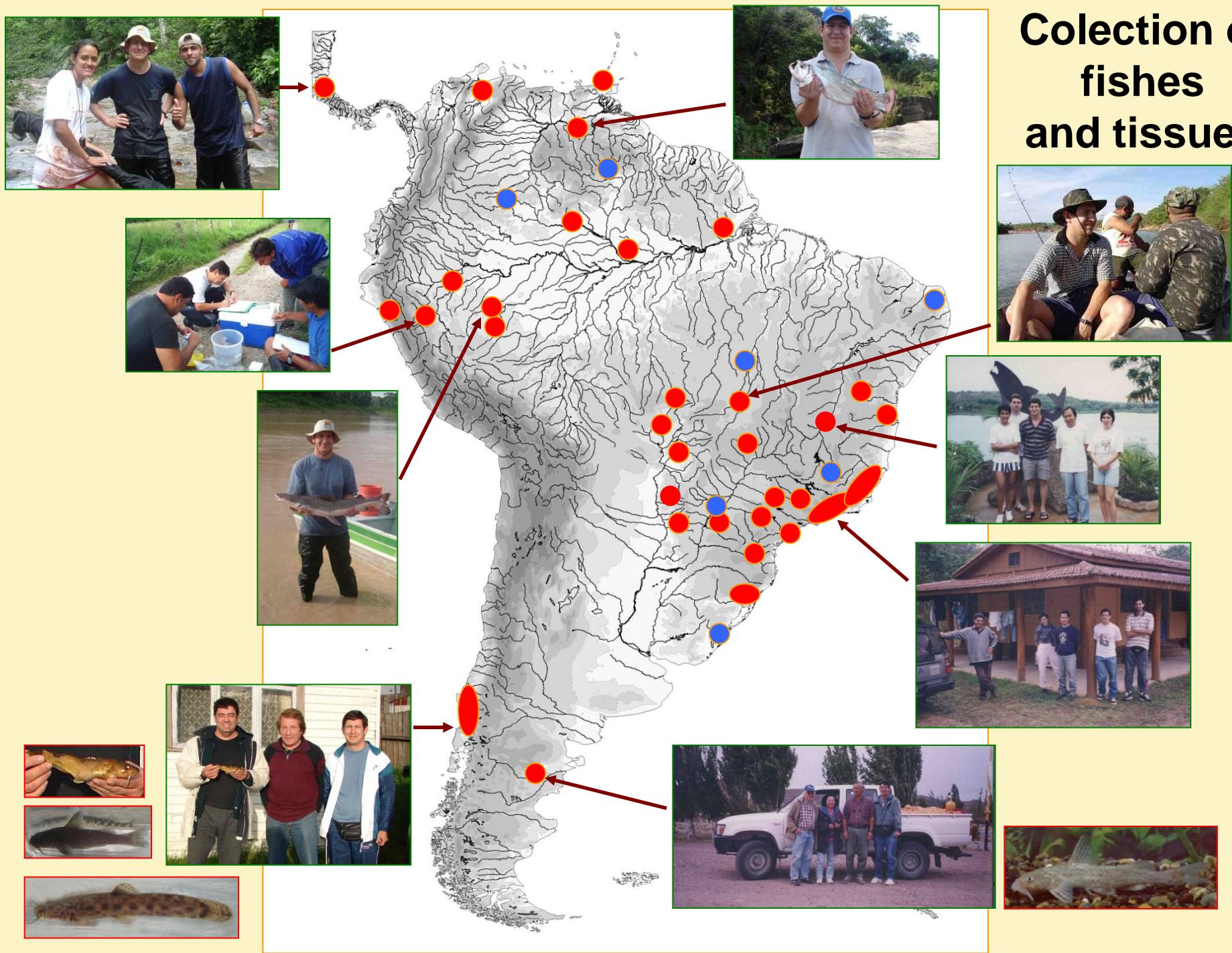
Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

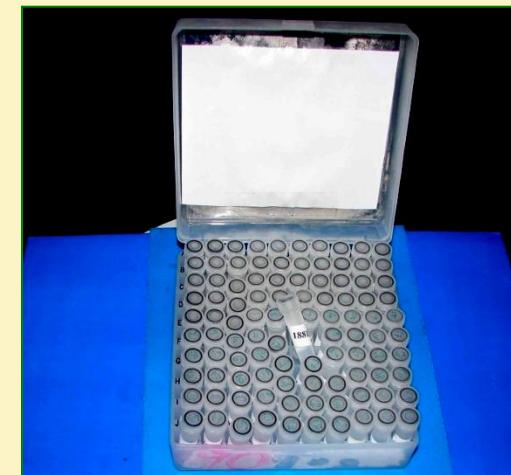


Molecular systematic and historical biogeography of the armored Neotropical
catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae)
Márcio Cesar Chiachio^a, Claudio Oliveira^a, Juan I. Montoya-Burgos^{b,*}

Colection of fishes and tissues



Fish and tissue collections



Fishes from Upper rio Paraná – a case study

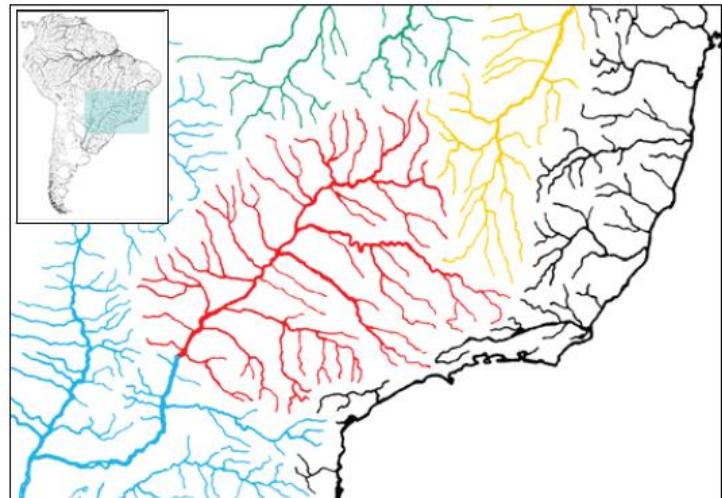
Diversidade da ictiofauna do Alto Rio Paraná: composição atual e perspectivas futuras

Francisco Langeani^{1,6}, Ricardo Macedo Corrêa e Castro², Osvaldo Takeshi Oyakawa³,

Oscar Akio Shibatta⁴, Carla Simone Pavanelli⁵ & Lilian Casatti¹

Biota Neotropica v7 (n3) – <http://www.biotaneotropica.org.br/v7n3/pt/abstract?article+bn03407032007>

Langeani, F., Castro, R.M.C., Oyakawa, O.T., Shibatta, O.A., Pavanelli, C.S. & Casatti, L. **Ichthyofauna diversity of the upper rio Paraná: present composition and future perspectives.** *Biota Neotrop.* Sep/Dez 2007 vol. 7, no. 3 <http://www.biotaneotropica.org.br/v7n3/pt/abstract?article+bn03407032007>. ISSN 1676-0603.



310 species/11 orders/38 families

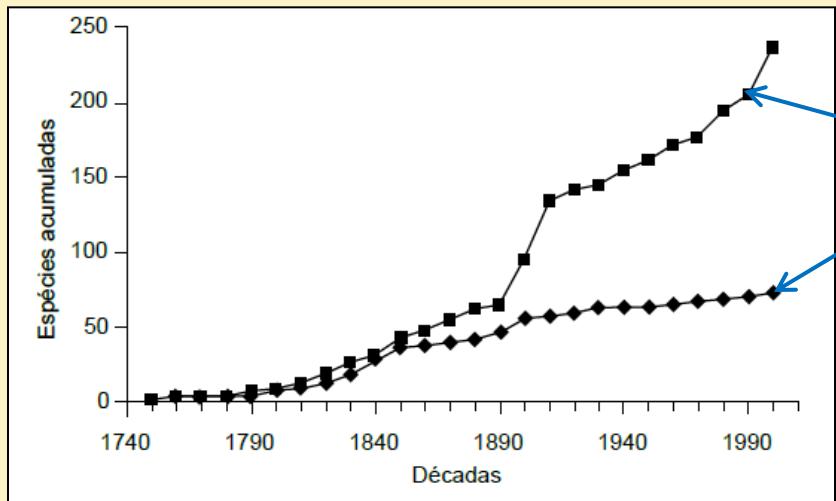
236 (76.1%) autochthonous

67 (21.6%) allochthonous

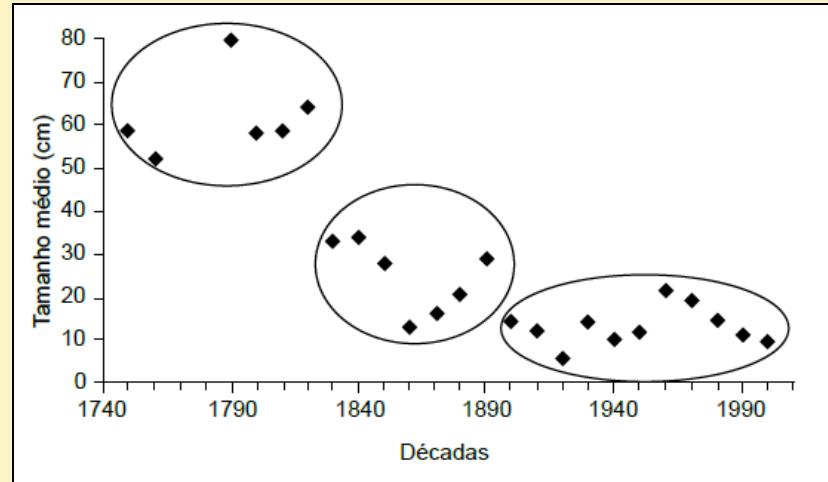
7 (2.3%) exotic

50 possible new species (~15%)

Fishes from Upper rio Paraná – a case study



Accumulated numbers of
autochthonous (squares) and
allochthonous plus exotic
(lozenges) by decade



Medium species size (cm) by decade

Fishes from Upper rio Paraná – a case study

Distribution patterns

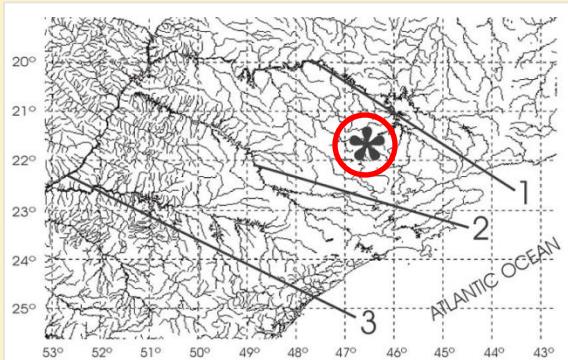
Endemism

Tricomycterus maracaya

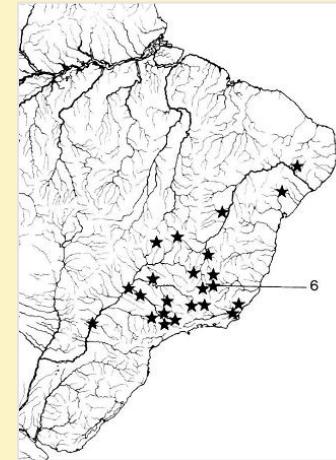


Wide distribution

Piabina argentea



Bockmann & Sazima, 2004



Vari & Harldi, 2001

Characteristics of Neotropical Fishes

- High diversity
- High description rates
- Distribution – endemism or wide distribution
- Many complexes and cryptic species
- Probably recent radiation

These particular characteristics make it very difficult to correctly identify many Neotropical fish species

How many species we have here?



M. oligolepis Amazonas



M. oligolepis Araguaia



M. oligolepis Paraguai



M. sanctaefilomenae Paraná



M. sanctaefilomenae Parnaíba



M. forestii La Plata Basin

DNA Barcode



THE ROYAL
SOCIETY

Received 29 July 2002
Accepted 30 September 2002
Published online 8 January 2003

Biological identifications through DNA barcodes

Paul D. N. Hebert*, Alina Cywinska, Shelley L. Ball
and Jeremy R. deWaard

Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Barcoding Animal Life: Cytochrome c Oxidase Subunit 1 Divergences among Closely Related Species

Paul D. N. Hebert; Sujeevan Ratnasingham; Jeremy R. deWaard

Proceedings: Biological Sciences, Vol. 270, Supplement: Biology Letters. (Aug. 7, 2003), pp. S96-S99.



How the DNA barcode can help us?

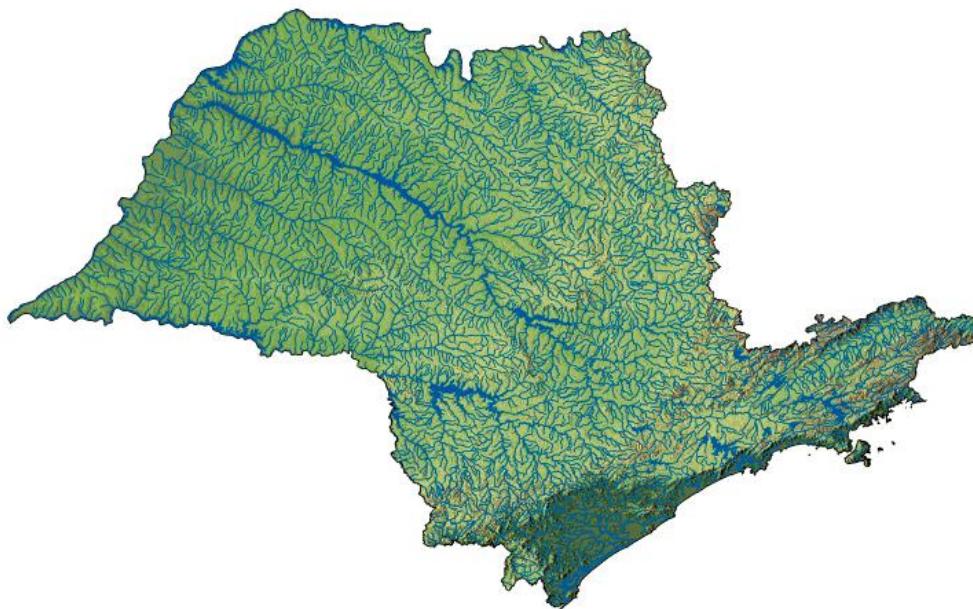
- Helping in the creation/expansion of new collections of fishes and tissues
- Testing hypotheses about species
- Generating information for rapid and precise species identification
- Allowing the comparison of faunas from different river basins

How the DNA barcode can help us?

a



b



Fishes from Upper Parana river

896 specimens/ 196 species/ 108 genera/ 31 families

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Fishes from Upper Parana River, Brazil [FUPR]

Summary Stats

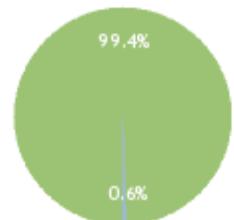
	Specimens (% complete)	Species (% complete)
COI-5P	896 / 1027 (87.2%)	196 / 200 (98%)

Sequence Data Report

Lacking Traces :	0
Sq's with stop codons :	0
Contaminated Sq's :	0

Specimen Data Report

Specimen records:	1027
Lacking geo reference:	116
Lacking photographs :	1027
Specimen Depositories:	



- Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes (1021)
- Universidade Estadual Paulista (6)

Marker(s)

Primary Marker : COI-5P

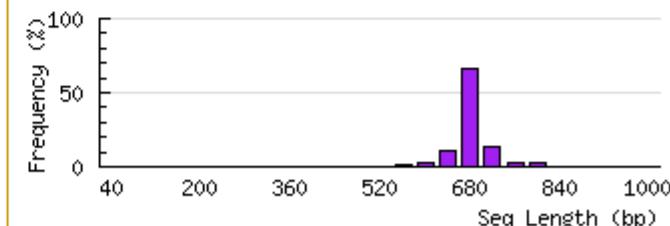
Sequence Quality Stats

	High (<1% Ns)	Medium (<2% Ns)	Low (<4% Ns)	Unreliable (>4% Ns)
COI-5P	100	0	0	0

Trace Quality Stats

	High(%)	Medium(%)	Low(%)	Failed(%)	Total
COI-5P	78.49	8.57	3.48	9.46	2041

Sequence Length Distribution - COI-5P



Project Growth : Specimen

Fishes from Brazilian Ribeira de Iguape River

311 specimens/ 53 species/ 39 genera/ 16 families

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Fishes from Brazilian Coastal Rivers part II [FBCRB]

Summary Stats

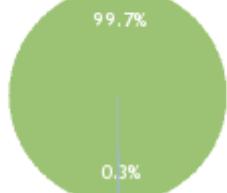
	Specimens (% complete)	Species (% complete)
COI-5P	311 / 383 (81.2%)	53 / 57 (93%)

Sequence Data Report

Lacking Traces :	0
Seqs with stop codons :	0
Contaminated Seqs :	0

Specimen Data Report

Specimen records:	383
Lacking geo reference:	47
Lacking photographs :	383
Specimen Depositories:	



- Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes (382)
- Universidade Federal Do Rio Grande Do Norte, Departamento de Biologia Celular e Genetica (1)

Marker(s)

Primary Marker : COI-5P

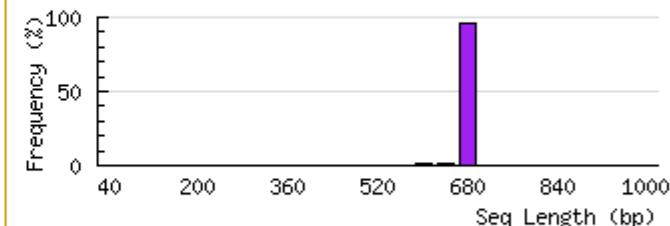
Sequence Quality Stats

	High (<1% Ns)	Medium (<2% Ns)	Low (<4% Ns)	Unreliable (>4% Ns)
COI-5P	100	0	0	0

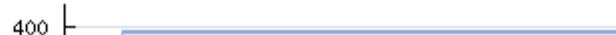
Trace Quality Stats

	High(%)	Medium(%)	Low(%)	Failed(%)	Total
COI-5P	81.09	7.42	2.38	9.11	714

Sequence Length Distribution - COI-5P



Project Growth : Specimen



Fishes from Brazilian Coastal Rivers

376 specimens/ 66 species/ 46 genera/ 19 families

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Fishes from Brazilian coastal rivers [FBCR]

Summary Stats

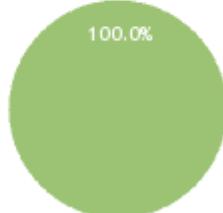
	Specimens (% complete)	Species (% complete)
COI-5P	376 / 539 (69.8%)	68 / 91 (74.7%)

Sequence Data Report

Lacking Traces :	0
Seqs with stop codons :	0
Contaminated Seqs :	0

Specimen Data Report

Specimen records:	539
Lacking geo reference:	508
Lacking photographs :	539
Specimen Depositories:	



Universidade Estadual
Paulista, Lab de
Biologia e Genetica de
Peixes (539)

Marker(s)

Primary Marker : COI-5P

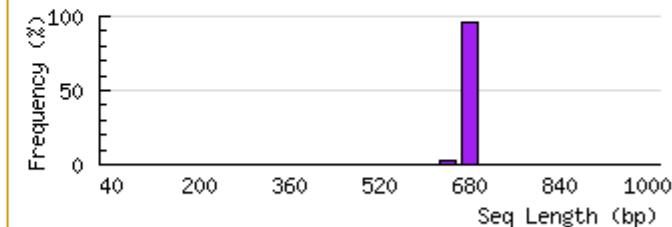
Sequence Quality Stats

	High (<1% Ns)	Medium (<2% Ns)	Low (<4% Ns)	Unreliable (>4% Ns)
COI-5P	100	0	0	0

Trace Quality Stats

	High(%)	Medium(%)	Low(%)	Failed(%)	Total
COI-5P	91.14	3.52	0.97	4.37	824

Sequence Length Distribution - COI-5P



Project Growth : Specimen

Fishes from Paraíba do Sul River

251 specimens/ 54 species/ 40 genera/ 17 families

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Fishes from Paraíba do Sul River, Brazil [FPSR]

Summary Stats

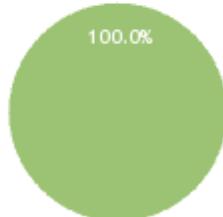
	Specimens (% complete)	Species (% complete)
COI-5P	251 / 292 (86%)	54 / 59 (91.5%)

Sequence Data Report

Lacking Traces :	0
Seqs with stop codons :	0
Contaminated Seqs :	0

Specimen Data Report

Specimen records:	292
Lacking geo reference:	12
Lacking photographs :	292
Specimen Depositories:	



- Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes (292)

Marker(s)

Primary Marker : COI-5P

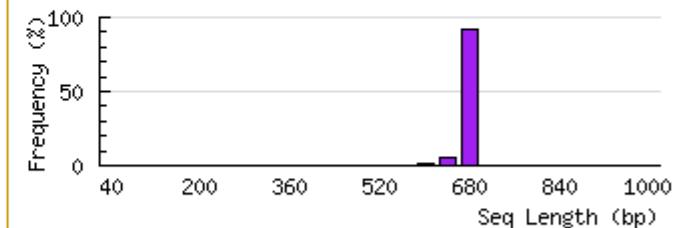
Sequence Quality Stats

	High (<1% Ns)	Medium (<2% Ns)	Low (<4% Ns)	Unreliable (>4% Ns)
COI-5P	100	0	0	0

Trace Quality Stats

	High(%)	Medium(%)	Low(%)	Failed(%)	Total
COI-5P	84.68	4.6	2.41	8.31	914

Sequence Length Distribution - COI-5P



Project Growth : Specimen

Marine Fishes from São Paulo

234 specimens/ 55 species/ 51 genera/ 36 families

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Marine Fishes from São Paulo, Brazil [MFSP]

Summary Stats

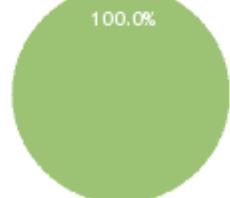
	Specimens (% complete)	Species (% complete)
COI-5P	234 / 342 (68.4%)	55 / 71 (77.5%)

Sequence Data Report

Lacking Traces :	0
Seqs with stop codons :	0
Contaminated Seqs :	0

Specimen Data Report

Specimen records:	342
Lacking geo reference:	50
Lacking photographs :	342
Specimen Depositories:	Universidade Estadual Paulista, Lab de Biologia e Genética de Peixes (342)



Marker(s)

Primary Marker : COI-5P

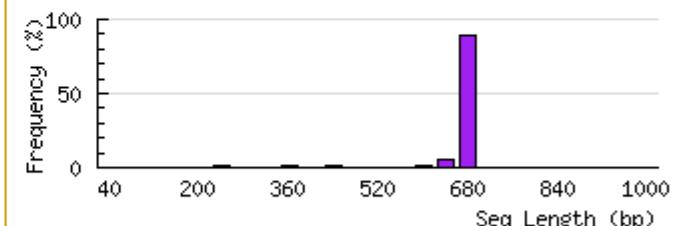
Sequence Quality Stats

	High (<1% Ns)	Medium (<2% Ns)	Low (<4% Ns)	Unreliable (>4% Ns)
COI-5P	100	0	0	0

Trace Quality Stats

	High(%)	Medium(%)	Low(%)	Failed(%)	Total
COI-5P	65.64	7.6	5.41	21.35	684

Sequence Length Distribution - COI-5P



Project Growth : Specimen

Records page

BOLD Systems - Record List - Windows Internet Explorer
http://www.boldsystems.org/views/recordlist.php?&

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Fishes from Upper Parana River, Brazil [FUPR]

Options List All Projects Back to Project Console Move Records to another Project Bibliography Submission Summary- Specimens, Localities, and Identifiers

Downloads Sequences Data Spreadsheet Specimen Labels Trace Files

Sequence Analysis Taxon ID Tree Distance Summary Sequence Composition Nearest Neighbor Summary Specimen Age vs Seq Length Alignment Browser Accumulation Curve

Identifications

Project Data : Select COI-5P: 899 Specimens: 1027

Identification ▼	Specimen Page ▼	Sequence Page ▼	COI-5P▼	Length [Ambig]	Contains	Extra Info ▼	Set
<input type="checkbox"/> Acestrorhynchus lacustris	LBP-15206	FUPR027-09	644 [On]	644		FAO-03	
<input type="checkbox"/> Acestrorhynchus lacustris	LBP-17213	FUPR026-09	636 [On]	636		FAO-03	
<input type="checkbox"/> Acestrorhynchus lacustris	LBP-17212	FUPR025-09	698 [On]	698		FAO-03	
<input type="checkbox"/> Acestrorhynchus lacustris	LBP-15174	FUPR024-09	637 [On]	637		FAO-03	
<input type="checkbox"/> Acestrorhynchus lacustris	LBP-15173	FUPR023-09	686 [On]	686		FAO-03	
<input type="checkbox"/> Ancistrus cirrhosus	LBP-34759	FUPR787-09	0	0			
<input type="checkbox"/> Ancistrus cirrhosus	LBP-34755	FUPR786-09	652 [On]	652			
<input type="checkbox"/> Ancistrus cirrhosus	LBP-34754	FUPR785-09	652 [On]	652			
<input type="checkbox"/> Ancistrus cirrhosus	LBP-34753	FUPR784-09	652 [On]	652			
<input type="checkbox"/> Ancistrus cirrhosus	LBP-34752	FUPR783-09	643 [On]	643			
<input type="checkbox"/> Ancistrus cirrhosus	LBP-35987	FUPR782-09	652 [On]	652			
<input type="checkbox"/> Ancistrus cirrhosus	LBP-35979	FUPR781-09	652 [On]	652			
<input type="checkbox"/> Ancistrus cirrhosus	LBP-35959	FUPR780-09	652 [On]	652			
<input type="checkbox"/> Apareiodon affinis	LBP-25976	FUPR402-09	0	0			
<input type="checkbox"/> Apareiodon affinis	LBP-26377	FUPR401-09	0	0			
<input type="checkbox"/> Apareiodon affinis	LBP-26376	FUPR400-09	[On]	[On]			
<input type="checkbox"/> Apareiodon affinis	LBP-26375	FUPR399-09	0	0			
<input type="checkbox"/> Apareiodon affinis	LBP-34671	FUPR398-09	617 [On]	617			
<input type="checkbox"/> Apareiodon ibitiensis	LBP-18635	FUPR433-09	652 [On]	652			

Done Internet | Protected Mode: Off Caixa de Entrada - ... Microsoft PowerPoint Coleção de Peixes Senhas [Modo de C... BOLD Systems - Rec... Desktop 21:16

Specimen page

BOLD Systems - Specimen Record - Windows Internet Explorer
http://www.boldsystems.org/views/specimenrecord.php?&

BOLD SYSTEMS v2.5 | Management & Analysis

Fishes from Upper Parana River, Brazil [FUPR]

Specimen Identifiers

Sample ID :	LBP-25638	Museum ID :	25638
Isolate / Field Num:	LBP2008030000	Collection Code :	LBP 4849
Donated By :		Deposited In :	Universidade Estadual Paulista, Lab de Biologia e Genetica de Peixes

[Edit Specimen](#)

Taxonomy

Identifier :	Claudio Oliveira
phylum :	Chordata
class :	Actinopterygii
order :	Siluriformes
family :	Pimelodidae
genus :	Steindachneridion
species :	Steindachneridion scriptum

Taxonomy Note :

Specimen Details

Voucher Type :	
Tissue Type :	
Extra Info :	
Sex :	
Reproduction :	S
Life Stage :	
Note :	

Collection Data

Collectors :	H Brandao
Date Collected :	01-Mar-2008
Country :	Brazil
State/Province:	Sao Paulo
Region/County :	Upper Parana Basin
Sector :	
Exact Site :	
Latitude :	-22.9037
Longitude :	-49.9834
Coord. Source :	
Coord.Accuracy :	
Elevation :	
Elevation Accuracy:	
Depth:	
Depth Accuracy:	



Photographs



Sequence page

BOLD Systems - Sequence Record - Windows Internet Explorer
http://www.boldsystems.org/views/sequencerecord.php?&

Barcode Identifiers

Barcode ID :	FUPR888-09	Sample ID :	LBP-25638
Identified As :	Steindachneridion scriptum		

COI-5P

Marker :	COI-5P	GenBank Accession :	
Last Updated :	2009-10-05	Translation Matrix :	Vertebrate Mitochondrial

Sequencing Runs

Run Date	Run Site	Direction Trace File	PCR primers	Seq Primer	Status
2009-09-22 18:22:03	Biodiversity Institute of Ontario	Forward FUPR888-09[C_FishF1t1,C_FishR1t1]_F.ab1	C_FishF1t1/C_FishR1t1	M13F	high qual
2009-09-22 19:48:09	Biodiversity Institute of Ontario	Reverse FUPR888-09[C_FishF1t1,C_FishR1t1]_R.ab1	C_FishF1t1/C_FishR1t1	M13R	med qual

[View Trace Files](#) [Download](#)

Nucleotide Sequence

Residues :	617	TTGGTACAGCTTAGCCTGTTAATTGGGGCAGAGCTAGCCCCAACCTGGCACTCTCTAGGGATGACCAAATCT
Comp. A :	168	ATAATGTCATCGTCACTGCTCATGCTGTAATAATTCTTATAGTAACTCTATTATGATCGGGAGGCTTGTG
Comp. G :	107	GAAACTGATTAGTCCCACTAATACTTCTGAGATAGCATTTCACGAAATGAACACATAAGCTTCTGAT
Comp. C :	158	TACTCCCCCACATTCTTACTACTACTTGCTCATCTGGGGTAGAAGCAGGAGCAGGGACGGGGTTGACCGTAT
Comp. T :	184	ATCGGCTCTTCGCGAAATCTTGACACGGCAGGGCTTCCGTAGACTTAACATTCTCCCTCCATTGGAG
Ambiguous :	0	GGGTCTCATCTAATCTAGGGCTATTAACCTTATTACACATTATTACATGAAACCTCCAGCTATTCAACAT

ACAAAACACCACTATTATTTGAGCCATTTTAACTACGGCGTACTACTCTACTGTCACTCCCAGTATTAGCTG

CAAGGCATTACATATATTAAACAGACGGAAATCTAAACTACATCTTGTGACCCAGCAGGGAGGAGACCAA

TTCCTTATCAACACCTT

[Clear Sequence](#)

Identity Sequence Using : [Full Database](#) [Species Database](#) [Ref Database](#)

Amino Acid Sequence

Residues :	217	GTALSLLIRAEAQPGTLLGDDQIYNVIVTAHAFMIFPMVMPIMIGGPGNWLVPMLIGAPIDMAFPMMNNMSFWL
		LPSPSFLLLASSGVVEAGAGTGIVYVPLLAGNLAHAGASVDLTIFPSLHLAGVSSILGAINFITIINMKPPAISQY
		QTPLFIWAILITAVLILSLFVLAAGITMLLTDRNLNITFFDPAGGGDPILYQHL-----

Publication

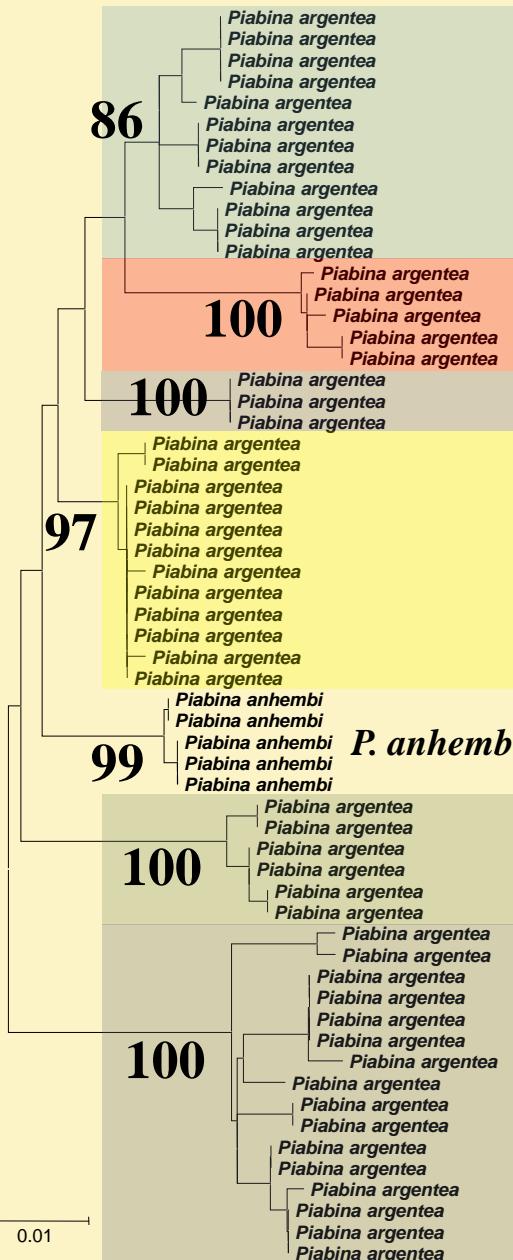
Illustrative Barcode

321 320 616

Sample Barcode Exam 1 IMC

Done [Internet](#) | Protected Mode: Off [75%](#)

Case study – *Piabina argentea*

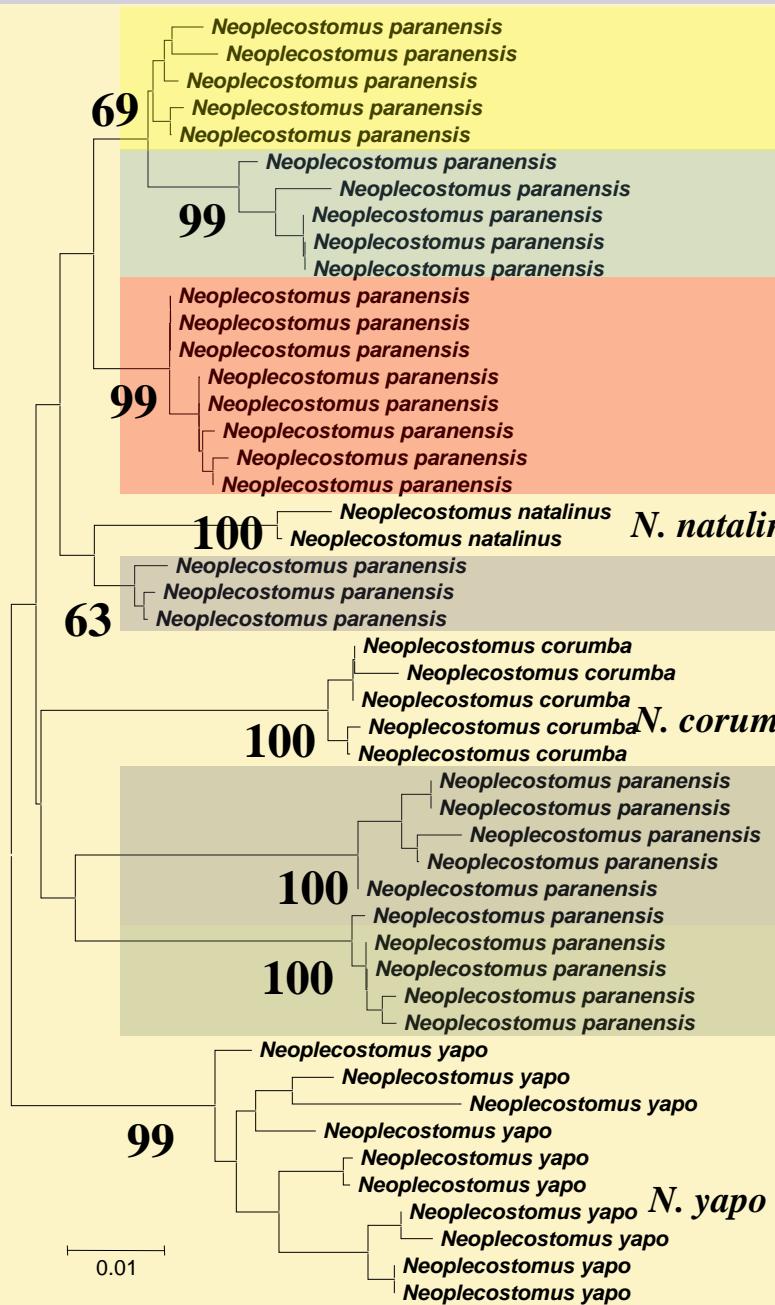


6 clusters (C)

K2P Divergence

	Min	Ave	Max
Intracluster	0%	0.4%	0.9%
Intercluster	2%	3.5%	5.6%
Congeneric species	2.2%	3.1%	4%
Intra vs. Inter		8.8 X	

Case study - *Neoplecostomus*



C1

C2

C3

C4

C5

C6

Neoplecostomus paranensis

6 clusters (C)

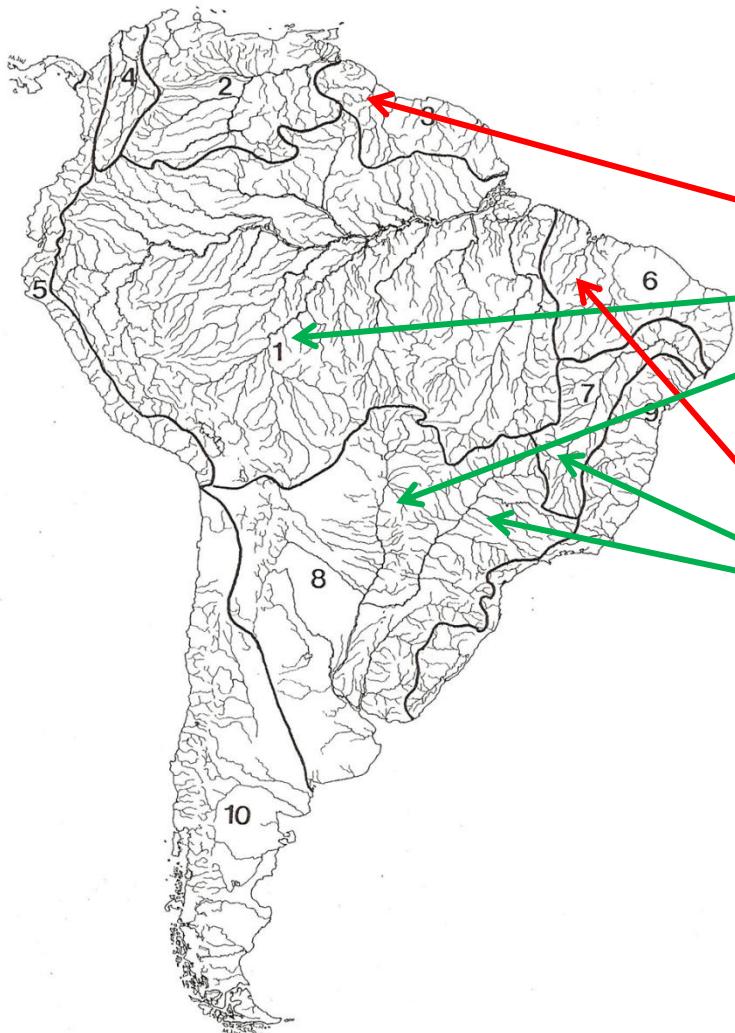


K2P Divergence

	Min	Ave	Max
Intracluster	0.3%	0.5%	0.7%
Intercluster	1.7%	4.9%	8.4%
Congeneric species	2.2%	5.5%	7.4%
Intra vs. Inter			8.9 X

We are describing 3 new species!

Case study - *Moenkhausia*



M. oligolepis

M. sanctaefilomenae



Different forms of *Moenkhausia*



M. oligolepis Amazonas



M. oligolepis Araguaia



M. oligolepis Paraguai

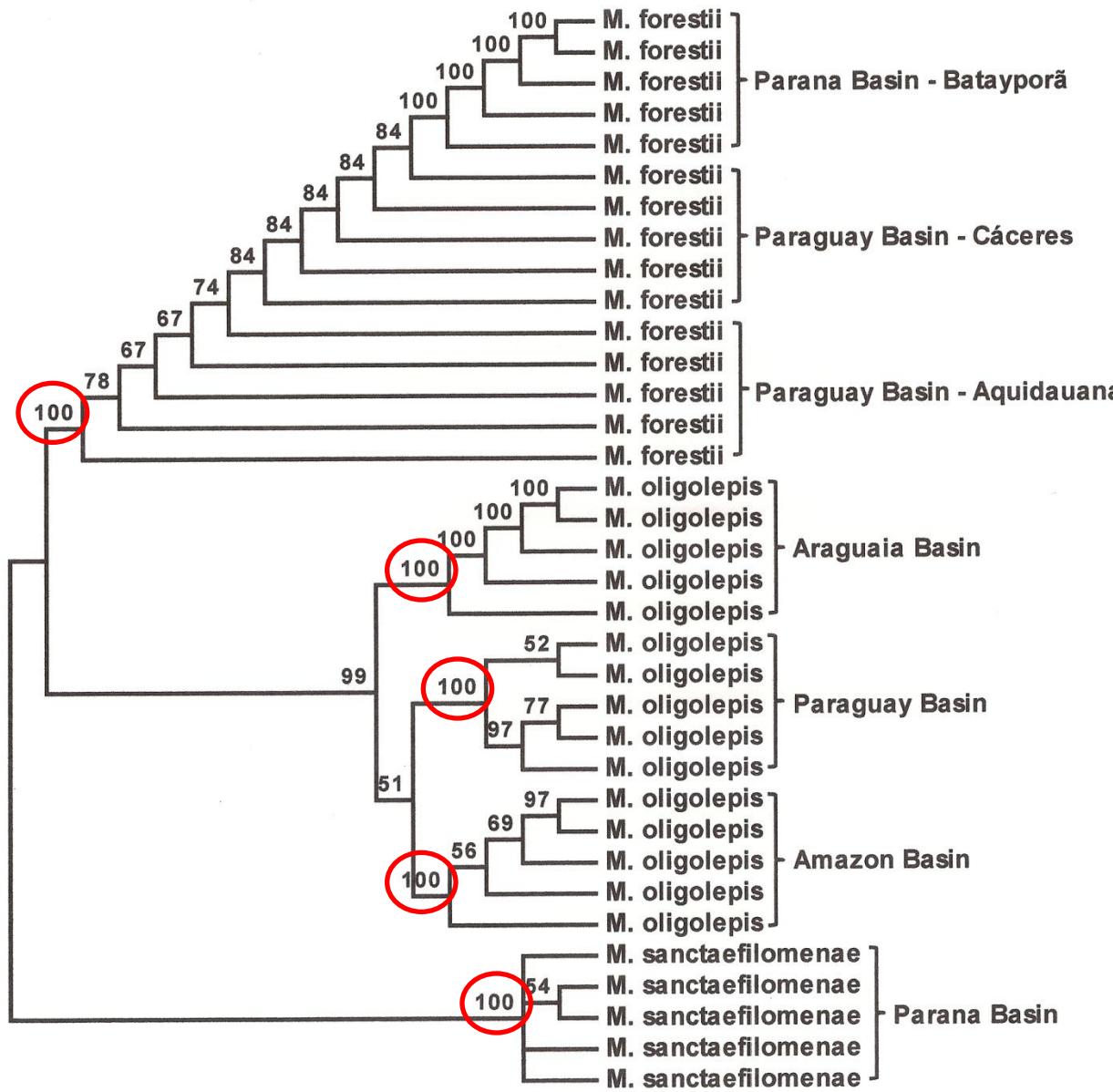


M. sanctaefilomenae Paraná



M. sp. La Plata Basin

Barcoding *Moenkhausia*



New species of *Moenkhausia* Eigenmann, 1903 (Characiformes: Characidae) with comments on the *Moenkhausia oligolepis* species complex

Ricardo C. Benine, Tatiane C. Mariguela and Claudio Oliveira



1

2

3

4

5

1 Moenkhausia sanctaefilomenae

Paraná Basin

EU177008 to EU177012

2 Moenkhausia oligolepis

Amazon Basin

EU177038 to EU177042

3 Moenkhausia oligolepis

Araguaia Basin

EU177013 to EU177017

4 Moenkhausia oligolepis

Paraguay Basin

EU177033 to EU177037

5 Moenkhausia forestii

Paraguay Basin - Aquidauana

EU177023 to EU177027

6 Moenkhausia forestii

Paraná Basin

EU177018 to EU177022

7 Moenkhausia forestii

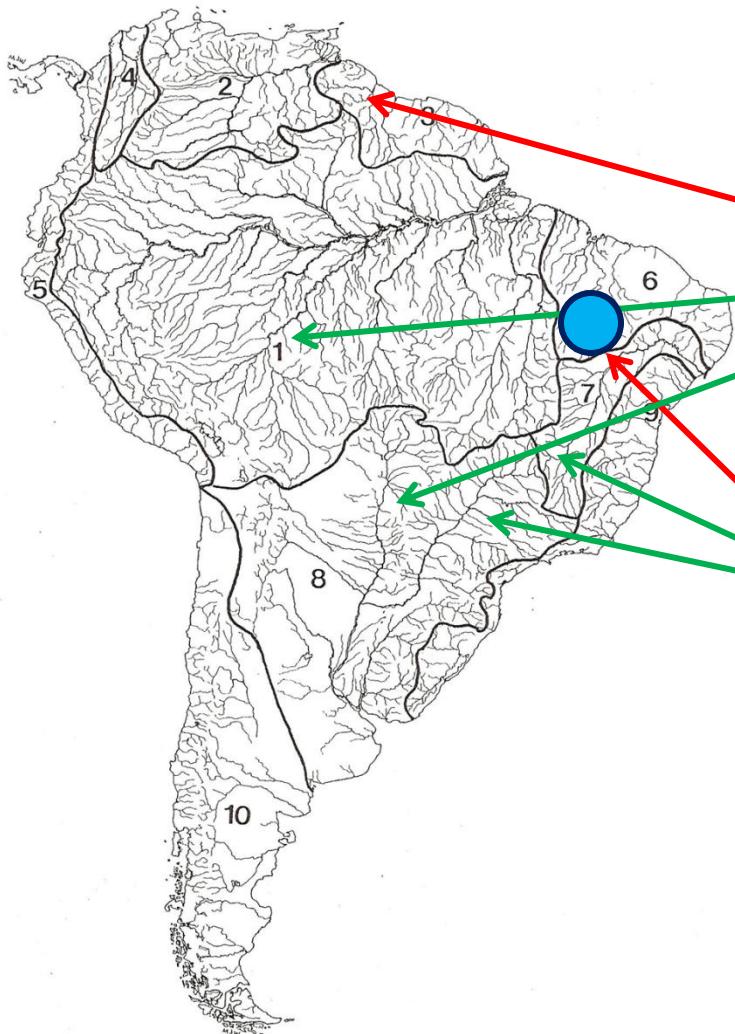
Paraguay Basin - Cáceres

EU177028 to EU177032

0.003±0.002				
0.190±0.019	0.010±0.003			
0.200±0.021	0.128±0.015	0.010±0.003		
0.184±0.018	0.109±0.013	0.139±0.016	0.014±0.003	
0.183±0.019	0.174±0.019	0.180±0.019	0.197±0.019	0.005±0.002
0.182±0.019	0.172±0.019	0.180±0.019	0.197±0.019	0.006±0.002
0.183±0.019	0.173±0.019	0.178±0.019	0.198±0.020	0.007±0.003

Moenkhausia forestii

Case study - *Moenkhausia*

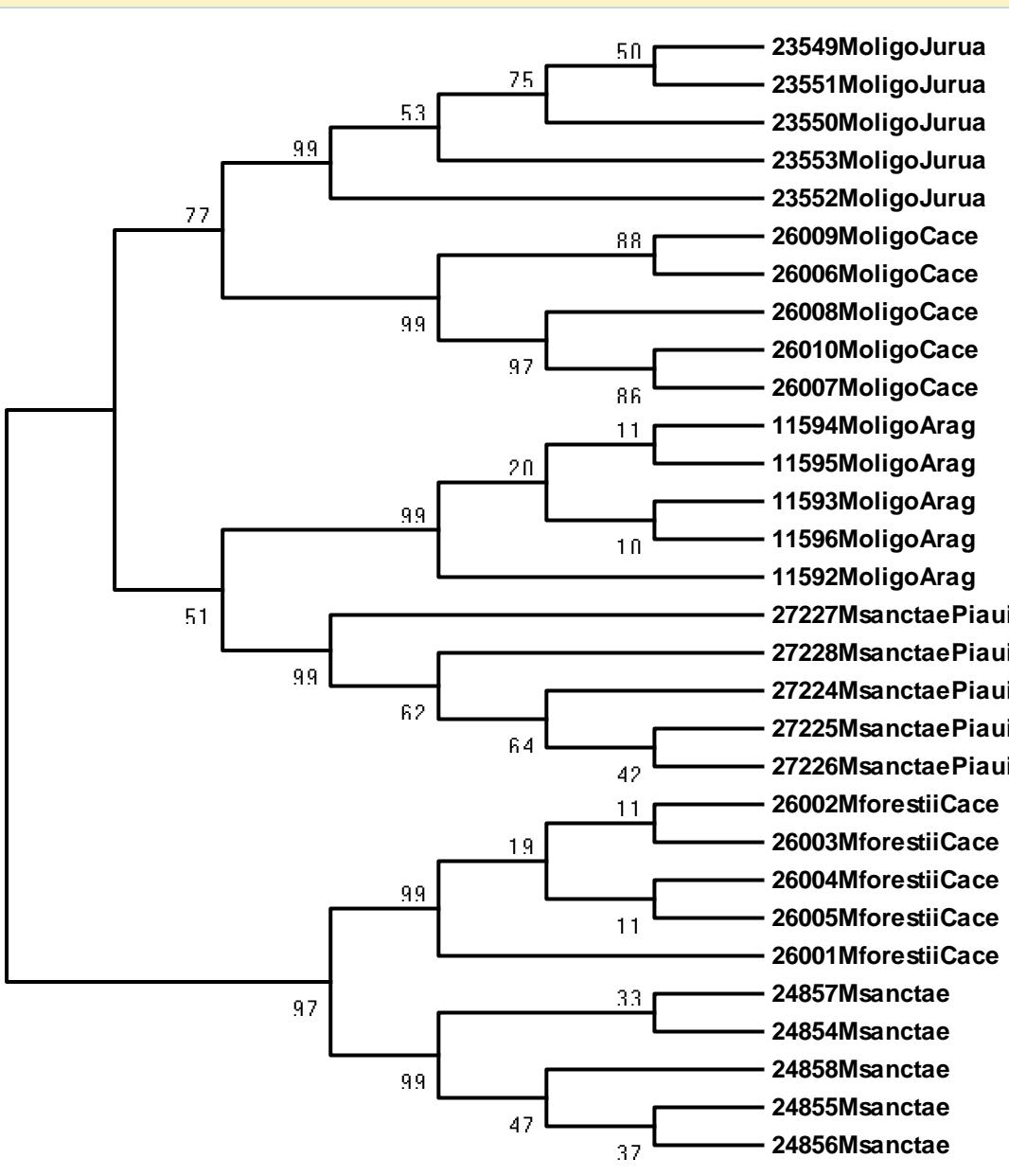


M. oligolepis

M. sanctaefilomenae



Three new species of *Moenkhausia*



M. oligolepis



M. sp. Paraguay River



M. sp. Araguaia River



M. sanctae filomenae



M. forestii



M. sp. Paraná River

Case study - *Mugil*



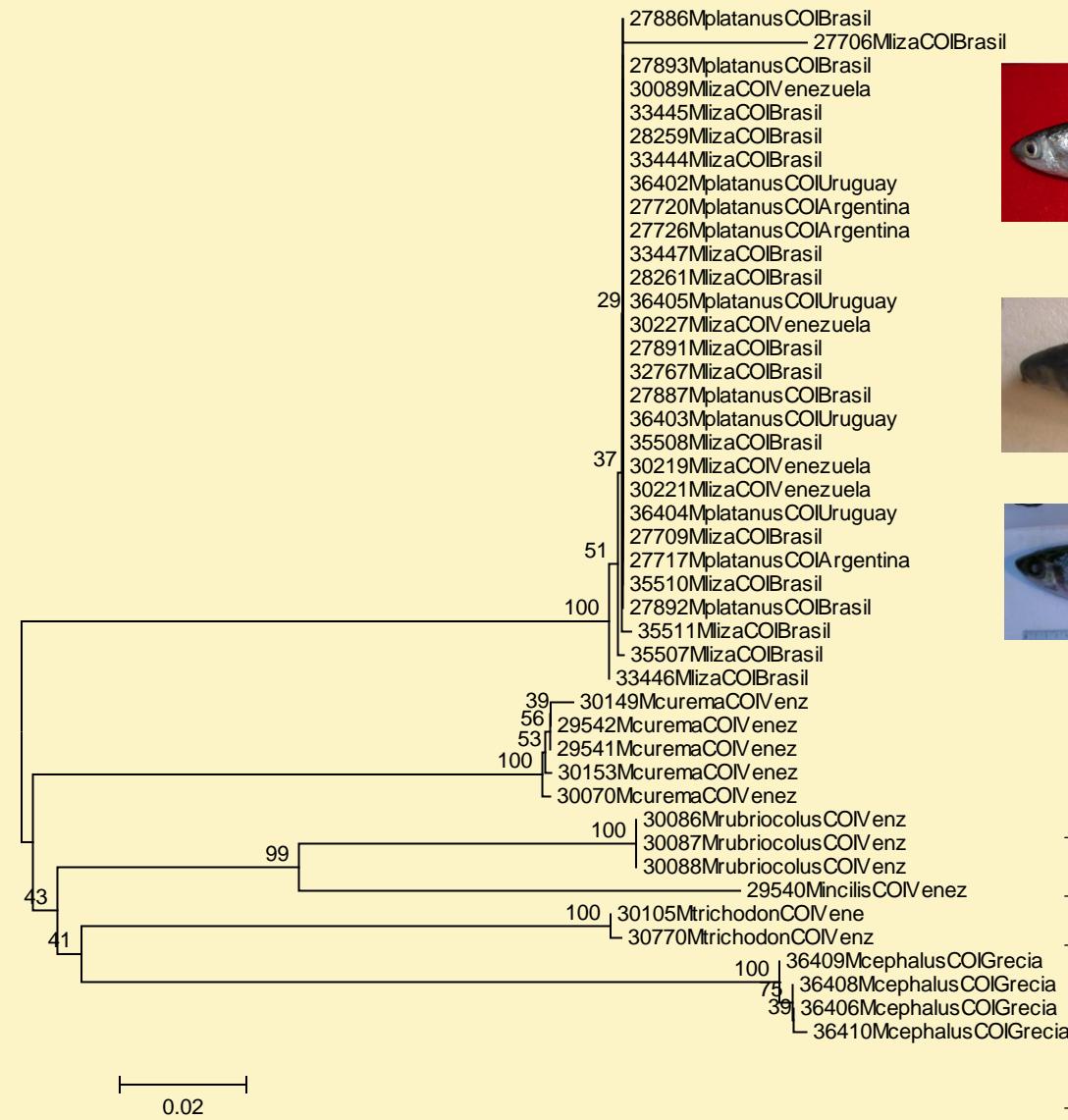
***Mugil liza* Valenciennes, 1836**



***Mugil platanus* Günther, 1880**



Case study - *Mugil*



Venezuela



Brazil



Argentina

Mugil liza

K2P Divergence - Groups

	Min	Ave	Max
Intragroups	0.1%	0.2%	0.3%
Intergroups	16.8%	19.4%	21.8%
Intra vs. Inter		97X	

Conclusion

→ Results have showed possible cryptic species in three widely distributed fish groups

- *Piabina argentea* Reinhardt, 1867 (6 species)
- *Neoplecostomus paranensis* Langeani, 1990 (6 species)

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- *Moenkhausia sp.* – three new species under description
- *Neoplecostomus sp.* – three new species under description

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- *Mugil platanus* = *Mugil liza*

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→ Results have showed possible synonym species names used for wide distributed species

- *Mugil platanus* = *Mugil liza*

→ Barcode really can improve our knowledge about species!

Collaborators:

Fausto Foresti

Luiz Henrique Garcia Pereira

Jefferson Monteiro Henriques

Gláucia Maria Garcia Maia

Zoila Raquel Siccha Ramirez

Ricardo Cardoso Benine

Tatiange Casagrande Mariguela

Robert Hanner

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