

**Supplementary material**

**Ecoregions and stream types help us understand ecological variability in Neotropical reference streams**

*Janaina Uchôa Medeiros Agra*<sup>A,E</sup>, *Raphael Ligeiro*<sup>B</sup>, *Diego Rodrigues Macedo*<sup>C</sup>,  
*Robert Mason Hughes*<sup>D</sup> and *Marcos Callisto*<sup>A</sup>

Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, Avenida Antonio Carlos 6627, CP 486, Belo Horizonte, MG, CEP 31270-901, Brazil.

<sup>B</sup>Universidade Federal do Pará, Instituto de Ciências Biológicas, Rua Augusto Corrêa 01, Guamá, Belém, PA, CEP 66075-110, Brazil.

<sup>C</sup>Universidade Federal de Minas Gerais, Instituto de Geociências, Departamento de Geografia, Avenida Antonio Carlos 6627, Belo Horizonte, MG, CEP 31270-901, Brazil.

<sup>D</sup>Amnis Opes Institute and Oregon State University, Department of Fisheries & Wildlife, 104 Nash Hall, Corvallis, OR 97331-3803, USA.

<sup>E</sup>Corresponding author. Email: [janaina.agra88@gmail.com](mailto:janaina.agra88@gmail.com)

**Table S1. Reference site locations**

Site code	Ecoregion	Longitude (UTM 23)	Latitude (UTM S)
SB08	Velhas Rainforest	654241	7752983
SB17	Velhas Rainforest	652080	7752668
SB18	Velhas Rainforest	650496	7753021
SB20	Velhas Rainforest	648570	7755323
SB21	Velhas Rainforest	655428	7748145
SB22	Velhas Rainforest	655369	7748151
SB24	Velhas Rainforest	654410	7752646
SB25	Velhas Rainforest	651422	7757002
SB26	Velhas Rainforest	650381	7755242
SB27	Velhas Rainforest	649680	7755078
SB28	Velhas Rainforest	651460	7757235
SB29	Velhas Rainforest	648156	7758988
SB30	Velhas Rainforest	648307	7755331
SB32	Velhas Rainforest	650421	7757203
SB33	Velhas Rainforest	648700	7759758
SB34	Velhas Rainforest	650125	7757183
SB35	Velhas Rainforest	650899	7754996
SB36	Velhas Rainforest	648182	7752886
SB37	Velhas Rainforest	652595	7754615
SB01	Caraça Grassland	649499	7761540
SB02	Caraça Grassland	647370	7761547
SB03	Caraça Grassland	649108	7761756
SB04	Caraça Grassland	651803	7760155
SB06	Caraça Grassland	646105	7762382
SB09	Caraça Grassland	647725	7762001
SB14	Caraça Grassland	646582	7764570
SB15	Caraça Grassland	646995	7763966
SB16	Caraça Grassland	647056	7761751
SB23	Caraça Grassland	647473	7761330
SB31	Caraça Grassland	648749	7762114

MACROINVERTEBRATES SAMPLE COLLECTION FORM - HEADWATERS													
SITE ID: _____										DATE: ___/___/___			
Coordinates:		Datum:		Zone/Area		Latitude North (Y)		Latitude West (X)					
UTM:													
Degrees, Minutes and Seconds or													
Decimal degrees													
Team:													
Contact:													
REACH-WIDE BENTHOS SAMPLE													
Transect:		A		B		C		D		E		F	
Sample ID													
Substrate /Code	Flow type /Code	Sub.	Flow	Sub.	Flow	Sub.	Flow	Sub.	Flow	Sub.	Flow	Sub.	Flow
Fine/Sand - FS Gravel - G Coarse - C Other: note in comments	Pool - P Glide - GL Riffle - RI Rapid - RA												

SLOPE AND BEARING FORM - HEADWATERS					
Transect	Slope (cm) (xx.x)	Bearing (0° - 359°)	Proport. (%)	First Supplemental	
				Bearing (0° - 359°)	Proportion (%)
E -> F					
D -> E					
C -> D					
B -> C					
A -> B					
Comments:					

STREAM DISCHARGE FORM			
Neutral Bouyant Object			
Float distance (m): _____			
Cross Sections on Float Reach:			
	Upper Section	Middle Section	Lower Section
Width (m)			
Depth 1 (cm)			
Depth 2			
Depth 3			
Depth 4			
Depth 5			
	Float 1	Float 2	Float 3
Float time (s):			

Fig. S1. Protocol of field data collection.

SITE ID: _____ Transect: A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> D <input type="checkbox"/> E <input type="checkbox"/> F <input type="checkbox"/>				DATE: ___/___/___			
Revised by: _____							
SUBSTRATE CROSS-SECTIONAL INFORMATION				BANK MEASUREMENTS			
	Depth (cm) xx.xx	Size class code	Embed. (0-100%)	Wetted Width xxx.x (m)			Bank Angle 0 - 360
Left				Bankfull Width xx.x (m)		Left	
Left center*				Bankfull Height xx.x (m)		Right	
Center				COMMENTS:			
Right center*							
Right							
<b>CANOPY COVER MEASUREMENTS</b>							
Densiometer (0 - 17 Max)							
Center up		Center left					
Center down		Center right					
Left*		Right*					
SUBSTRATE SIZE CLASS CODE			Embed. %				
RS = Bedrock (Smooth) (Larger than a car)			0				
RR = Bedrock (Rough) (Larger than a car)			0				
RC = Concrete / asphalt							
XB = Large boulder (1 to 4 m)							
CB = Cobble (64 to 250 mm)							
GC = Course Gravel (16 to 64 mm)							
GF = Fine Gravel (2 to 16 mm)							
SA = Sand (0.06 to 2 mm)			100				
FN = Fine (Silt/Clay/Muck)			100				
HP = Hardpan (Consolidated fine substrate)			0				
WD = Wood (any size)							
OT = Other (any size)							

\*For streams over 3.5 m width

**Fig. S1.** (Cont.)

PHYSICAL HABITAT FORM – HEADWATERS											
SITE ID: _____		Transect: A-B <input type="checkbox"/> B-C <input type="checkbox"/> C-D <input type="checkbox"/> D-E <input type="checkbox"/> E-F <input type="checkbox"/>			DATE: ___/___/___						
THALWEG PROFILE											
Station	Thalweg depth (cm) (xxx)	Soft/small sediment < gravel	Channel unit code	Substrate at station 2*	Left	Center	Right	Wetted width (m) (xx.x)			
0		Y N		Comments:							
1		Y N									
2*		Y N									
3		Y N									
4		Y N									
<b>VISUAL RIPARIAN ESTIMATES</b>		0 = Absent (0%) 1 = Sparse (<10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = Very Heavy (>75%)			<b>CHANNEL UNIT CODE</b> PP – Pool GL = Glide		RI = Riffle RA = Rapid CA = Cascade FA = Falls DR = Dry Channel				
		Riparian Vegetation Cover	Left Bank				Right Bank		<b>BENTHOS HABITAT</b>		
		Canopy (>5 m height)			Filamentous Algae		0 1 2 3 4				
Big trees (Trunk > 0.3 m DBH)		0 1 2 3 4		0 1 2 3 4		Macrophytes		0 1 2 3 4			
Small trees (Trunk < 0.3 m DBH)		0 1 2 3 4		0 1 2 3 4		Woody Debris > 0.3 m (BIG)		0 1 2 3 4			
		Understory (0.5 to 5 m height)			Brush/Woody Debris <0.3 m		0 1 2 3 4				
Woody Shrubs & Saplings		0 1 2 3 4		0 1 2 3 4		Leaf Litter		0 1 2 3 4			
Non-Woody Herbs, Grasses & Forbs		0 1 2 3 4		0 1 2 3 4		Undercut Banks		0 1 2 3 4			
		Ground Cover (<0.5 m height)			Boulders		0 1 2 3 4				
Woody Shrubs & Saplings		0 1 2 3 4		0 1 2 3 4		Artificial Structures		0 1 2 3 4			
Non-Woody Herbs, Grasses & Forbs		0 1 2 3 4		0 1 2 3 4		<b>COMMENTS:</b>					
Barren, Bare Dirt or Duff		0 1 2 3 4		0 1 2 3 4							
<b>HUMAN INFLUENCE</b>		0 = not present P = dist >10 m C = within <10 m B = On bank			LEFT BANK				RIGHT BANK		
Wall/Revetment/Riprap/Dam		0 P C B		0 P C B							
Buildings		0 P C B		0 P C B							
Pavement/Cleared Lot		0 P C B		0 P C B							
Road/Railroad		0 P C B		0 P C B							
Pipes (Inlet/Outlet)		0 P C B		0 P C B							
Landfill/Trash		0 P C B		0 P C B							
Park/Lawn		0 P C B		0 P C B							
Row Crops		0 P C B		0 P C B							
Pasture/Range/Hay Field		0 P C B		0 P C B							
Logging Operations		0 P C B		0 P C B							
Mining Activity		0 P C B		0 P C B							

Fig. S1. (Cont.)

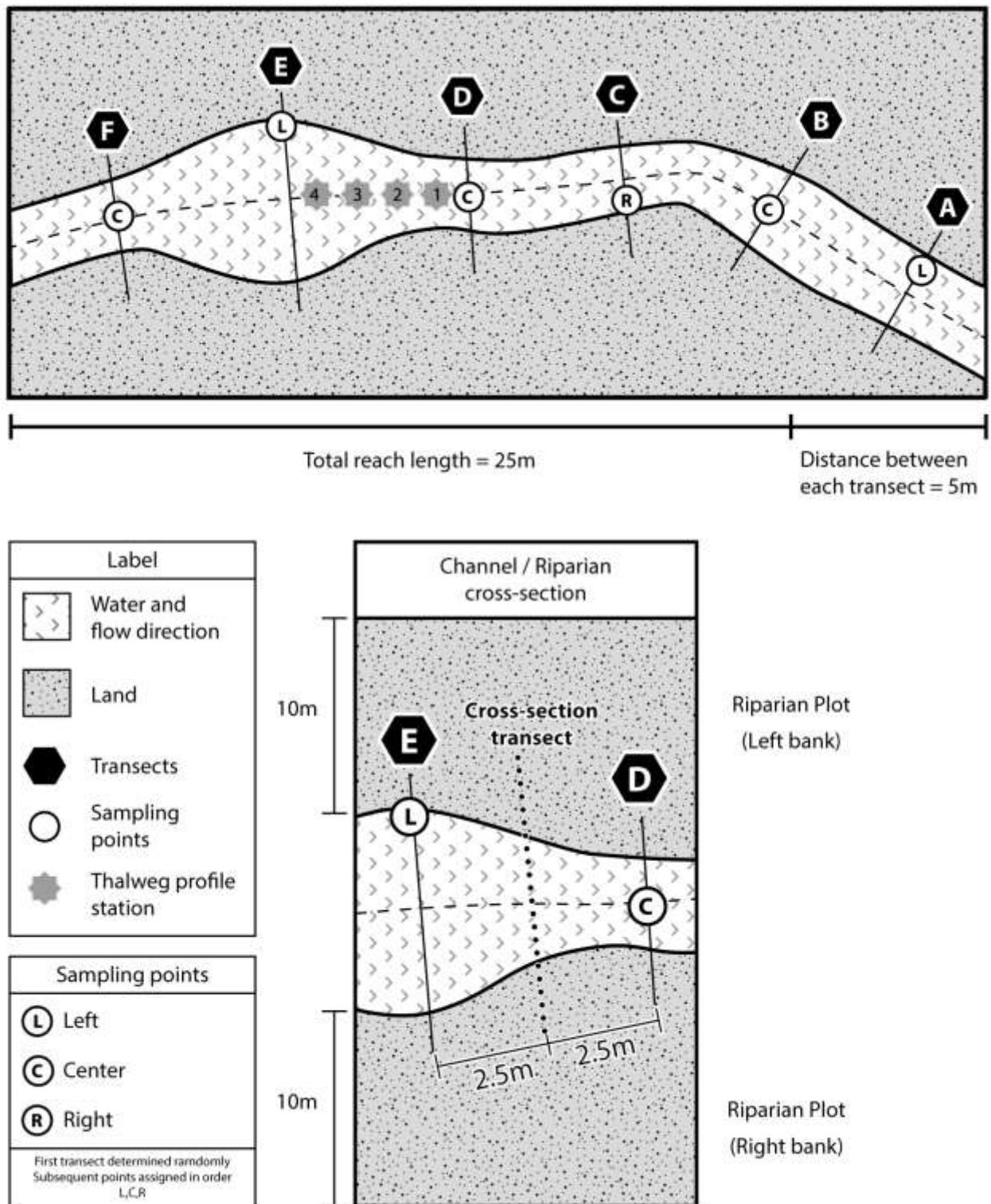


Fig. S1. (Cont.)

**Table S2. Macroinvertebrate taxa abundances, relative abundances (%) and frequency per stream type**

Indicator taxa are in bold

Taxa	Caraça Grassland			Velhas Rainforest					
	Type 1			Type 2			Type 3		
	Abundance	Relative abundance (%)	Frequency (%)	Abundance	Relative abundance (%)	Frequency (%)	Abundance	Relative abundance (%)	Frequency (%)
<b>Diptera</b>									
Chironomidae	2529	46.4	100	3320	36.7	100	880	21.2	100
Simuliidae	174	3.2	90.9	270	3	77.8	179	4.3	80
Ceratopogonidae	132	2.4	90.9	190	2.1	100	47	1.1	90
Tipulidae	124	2.3	100	226	2.5	100	112	2.7	80
Dixidae	4	0.1	36.4	18	0.2	55.6	1	0	10
Culicidae	4	0.1	27.3	2	0	22.2	0	0	0
Tabanidae	15	0.3	36.4	35	0.4	77.8	6	0.1	30
Empididae	12	0.2	45.5	11	0.1	66.7	13	0.3	50
Muscidae	1	0	9.1	0	0	0	0	0	0
Psychodidae	0	0	0	3	0	11.1	0	0	0
<b>Lepidoptera</b>									
Pyralidae	11	0.2	36.4	12	0.1	44.4	5	0.1	20
<b>Ephemeroptera</b>									
Caenidae	1	0	9.1	162	1.8	22.2	3	0.1	20
Leptohyphidae	1	0	9.1	1245	13.7	88.9	100	2.4	90
Euthyplociidae	51	0.9	45.5	11	0.1	22.2	34	0.8	60
Baetidae	209	3.8	100	105	1.2	100	237	5.7	100
Leptophlebiidae	814	14.9	90.9	171	1.9	100	448	10.8	100
<b>Odonata</b>									
Calopterygidae	9	0.2	36.4	10	0.1	55.6	55	1.3	50
Megapodagrionidae	26	0.5	54.5	57	0.6	77.8	51	1.2	100
Perilestidae	12	0.2	27.3	3	0	11.1	0	0	0
Coenagrionidae	48	0.9	72.7	32	0.4	66.7	63	1.5	90
Aeshnidae	16	0.3	54.5	6	0.1	33.3	10	0.2	30
Gomphidae	1	0	9.1	74	0.8	88.9	30	0.7	60
Libellulidae	16	0.3	63.6	37	0.4	88.9	41	1	80
Corduliidae	3	0.1	27.3	13	0.1	44.4	1	0	10
Protoneuridae	3	0.1	9.1	0	0	0	0	0	0

Taxa	Caraça Grassland			Velhas Rainforest					
	Type 1			Type 2			Type 3		
	Abundance	Relative abundance (%)	Frequency (%)	Abundance	Relative abundance (%)	Frequency (%)	Abundance	Relative abundance (%)	Frequency (%)
Plecoptera									
Perlidae	160	2.9	81.8	159	1.8	100	229	5.5	100
Gripopterygidae	100	1.8	81.8	208	2.3	100	153	3.7	100
Hemiptera									
Belostomatidae	0	0	0	7	0.1	22.2	0	0	0
Pleidae	0	0	0	20	0.2	22.2	0	0	0
<b>Notonectidae</b>	<b>17</b>	<b>0.3</b>	<b>45.5</b>	1	0	11.1	0	0	0
Helotrephidae	0	0	0	9	0.1	11.1	0	0	0
Naucoridae	7	0.1	27.3	14	0.2	22.2	0	0	0
Mesoveliidae	1	0	9.1	0	0	0	0	0	0
Noteridae	4	0.1	27.3	0	0	0	0	0	0
Veliidae	32	0.6	81.8	10	0.1	55.6	12	0.3	40
Coleoptera									
<b>Hydrophilidae</b>	9	0.2	27.3	<b>20</b>	<b>0.2</b>	<b>88.9</b>	5	0.1	20
Dytiscidae	12	0.2	54.5	17	0.2	44.4	0	0	0
Staphylinidae	1	0	9.1	3	0	22.2	0	0	0
Scirtidae	5	0.1	27.3	23	0.3	44.4	18	0.4	30
<b>Psephenidae</b>	<b>39</b>	<b>0.7</b>	<b>54.5</b>	0	0	0	0	0	0
<b>Lutrochidae</b>	33	0.6	27.3	<b>87</b>	<b>1</b>	<b>88.9</b>	11	0.3	50
Elmidae	224	4.1	90.9	576	6.4	100	419	10.1	100
Dryopidae	3	0.1	27.3	9	0.1	55.6	12	0.3	20
<b>Ptilodactylidae</b>	12	0.2	27.3	<b>70</b>	<b>0.8</b>	<b>88.9</b>	3	0.1	20
Gyrinidae	1	0	9.1	0	0	0	1	0	10
Megaloptera									
Corydalidae	14	0.3	36.4	13	0.1	66.7	34	0.8	70
Trichoptera									
Philopotamidae	20	0.4	54.5	40	0.4	55.6	6	0.1	30
Polycentropodidae	99	1.8	90.9	197	2.2	77.8	34	0.8	60
Hydrobiosidae	5	0.1	18.2	1	0	11.1	0	0	0
Glossosomatidae	2	0	9.1	8	0.1	33.3	4	0.1	30
Hydropsychidae	142	2.6	54.5	74	0.8	88.9	201	4.8	100
Helicopsychidae	149	2.7	81.8	1	0	11.1	32	0.8	50
Leptoceridae	20	0.4	27.3	198	2.2	88.9	73	1.8	60
Calamoceratidae	34	0.6	45.5	218	2.4	88.9	98	2.4	80



Taxa	Caraça Grassland			Velhas Rainforest					
	Type 1			Type 2			Type 3		
	Abundance	Relative abundance (%)	Frequency (%)	Abundance	Relative abundance (%)	Frequency (%)	Abundance	Relative abundance (%)	Frequency (%)
Ecnomidae	0	0	0	1	0	11.1	0	0	0
<b>Odontoceridae</b>	16	0.3	27.3	<b>137</b>	<b>1.5</b>	<b>88.9</b>	5	0.1	20
Amphipoda									
Hyalellidae	0	0	0	348	3.8	55.6	171	4.1	30
Tricladida									
Planariidae	6	0.1	27.3	49	0.5	66.7	13	0.3	70
Oligochaeta	59	1.1	81.8	332	3.7	100	110	2.6	100
Hydracarina	1	0	9.1	7	0.1	44.4	13	0.3	50
Bivalvia	8	0.1	9.1	179	2	88.9	182	4.4	60
Collembola	2	0	18.2	6	0.1	66.7	4	0.1	30
Hirudinea	0	0	0	2	0	11.1	0	0	0
Total	5453	100	-	9057	100	-	4159	100	-