

TABLE S1 | Identification numbers, species, vouchers, collection sites, geographic coordinates, BOLD, BIN and GenBank accession numbers for *Astyanax* species from the Paraguaçu basin analyzed in this study. UFBA, Museu de História Natural da Bahia, Universidade Federal da Bahia; LISDEBE, Laboratório de Ictiologia Sistemática, Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos; 1- COI, *Cytochrome Oxidase Subunit 1*; 2- Cytb, *Cytochrome b*; 3- S7, 1st intron of the S7 ribosomal protein gene.

| ID | Collection | | | | | Accession numbers | | | |
|--------|------------|---------------------------------|---------------------|-------------------|--------------|-------------------|------------------------------|-------------------|-------------------------|
| | Sample | Species/Vouchers | Sub-basin | Site (river) | Latitude | Longitude | (COI/BIN) ¹ | Cytb ² | S7 (A1/A2) ³ |
| 8095-1 | | <i>A. brucutu</i> /UFBA 08204 | Santo Antônio River | Pratinha River | 12°21'13"S | 41°32'51"W | ASTBA090-19/ BOLD:AAC5910 | MN061564 | MN061500/ MN061501 |
| 8095-2 | | <i>A. brucutu</i> | Santo Antônio River | Pratinha River | 12°21'13"S | 41°32'51"W | ASTBA091-19/ BOLD:AAC5910 | MN061565 | MN061502/ MN061503 |
| 8095-4 | | <i>A. brucutu</i> | Santo Antônio River | Pratinha River | 12°21'13"S | 41°32'51"W | ASTBA092-19/ BOLD:AAC5910 | MN061566 | MN061504/ MN061505 |
| 8463-1 | | <i>A. epiagos</i> /UFBA 08463 | Jacuípe River | Ferro Doido River | 11°20'27.2"S | 41°04'27.8"W | ASTBA085-19/ BOLD:AAC5910 | MN061558 | MN061508/ MN061509 |
| 8463-2 | | <i>A. epiagos</i> | Jacuípe River | Ferro Doido River | 11°20'27.2"S | 41°04'27.8"W | ASTBA086-19/ BOLD:AAC5910 | MN061559 | MN061510/ MN061511 |
| 8463-3 | | <i>A. epiagos</i> | Jacuípe River | Ferro Doido River | 11°20'27.2"S | 41°04'27.8"W | ASTBA087-19/ BOLD:AAC5910 | MN061560 | MN061512/ MN061513 |
| 8463-4 | | <i>A. epiagos</i> | Jacuípe River | Ferro Doido River | 11°20'27.2"S | 41°04'27.8"W | ASTBA088-19/ BOLD:AAC5910 | MN061561 | MN061514/ MN061515 |
| 8463-5 | | <i>A. epiagos</i> | Jacuípe River | Ferro Doido River | 11°20'27.2"S | 41°04'27.8"W | ASTBA089-19/ BOLD:AAC5910 | MN061562 | MN061516/ MN061517 |
| RPR1 | | <i>A. lorien</i> /UFBA 06589 | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA065-17/ BOLD:AAC5910 | KR007382 | - |
| RPR3 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA066-17/ BOLD:AAC5910 | KR007383 | KR007555/ KR007556 |
| RPR4 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA067-17/ BOLD:AAC5910 | KR007385 | MN061556/ MN061557 |
| RPR5 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA068-17/ BOLD:AAC5910 | - | - |
| RPR6 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA069-17/ BOLD:AAC5910 | KR007386 | - |
| RPR7 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA070-17/ BOLD:AAC5910 | KR007387 | KR007557/ KR007558 |
| RPR8 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA071-17/ BOLD:AAC5910 | KR007388 | KR007559/ KR007560 |
| RPR9 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA072-17/ BOLD:AAC5910 | - | KR007561/ KR007562 |
| RPR10 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA073-17/ BOLD:AAC5910 | KR007389 | KR007563/ KR007564 |
| RPR11 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA074-17/ BOLD:AAC5910 | KR007390 | KR007565/ KR007566 |
| RPR13 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA076-17/ BOLD:AAC5910 | KR007392 | KR007567/ KR007568 |
| RPR15 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | - | MN061573 | KR007569/ KR007570 |
| RPR20 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA082-17/ BOLD:AAC5910 | - | KR007571/ KR007572 |
| RPR21 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA083-17/ BOLD:AAC5910 | KR007399 | KR007573/ KR007574 |
| RPR22 | | <i>A. lorien</i> | Santo Antônio River | Preto River | 12°36'12"S | 41°31'29"W | ASTBA084-17/ BOLD:AAC5910 | KR007400 | KR007575/ KR007576 |
| RCB1 | | <i>A. rupestris</i> /UFBA 06590 | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19'5.8"W | ASTBA001-17/ BOLD:ADI2769 | KR007312 | MN061524/ MN061525 |
| RCB2 | | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA002-17/ BOLD:ADI2769 | KR007313 | KR007485/ KR007486 |
| RCB3 | | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA003-17/ BOLD:ADI2769 | KR007314 | KR007487/ KR007488 |
| RCB4 | | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA004-17/ BOLD:ADI2769 | KR007315 | KR007489/ KR007490 |
| RCB5 | | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA005-17/ BOLD:ADI2769 | KR007316 | KR007491/ KR007492 |
| RCB6 | | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA006-17/ BOLD:ADI2769 | KR007317 | KR007493/ KR007494 |
| RCB8 | | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA008-17/ BOLD:ADI2769 | KR007319 | - |



TABLE S1 | (Continued)

| ID | Collection | | | | | Accession numbers | | |
|--------|---------------------------------------|---------------|-----------------|--------------|--------------|------------------------------|-------------------|-------------------------|
| Sample | Species/Vouchers | Sub-basin | Site (river) | Latitude | Longitude | (COI/BIN) ¹ | Cytb ² | S7 (A1/A2) ³ |
| RCB9 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA009-17/ BOLD:ADI2769 | KR007320 | KR007495/ KR007496 |
| RCB10 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA010-17/ BOLD:ADI2769 | KR007321 | KR007497/ KR007498 |
| RCB11 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA011-17/ BOLD:ADI2769 | KR007323 | KR007499/ KR007500 |
| RCB12 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA012-17/ BOLD:ADI2769 | KR007323 | MN061526/ MN061527 |
| RCB15 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA015-17/ BOLD:ADI2769 | KR007326 | MN061528/ MN061529 |
| RCB16 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA016-17/ BOLD:ADI2769 | KR007327 | - |
| RCB18 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA018-17/ BOLD:ADI2769 | KR007329 | KR007501/ KR007502 |
| RCB19 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA019-17/ BOLD:ADI2769 | KR007330 | - |
| RCB20 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA020-17/ BOLD:ADI2769 | KR007331 | MN061532/ MN061533 |
| RCB21 | <i>A. rupestris</i> | Piabas River | Coisa Boa River | 12°53'46.5"S | 41°19' 5.8"W | ASTBA021-17/ BOLD:ADI2769 | KR007332 | KR007503/ KR007504 |
| 8510-1 | <i>A. rupestris</i> | Cumbuca River | Cumbuca River | 13°00'36.9"S | 41°12'14.0"W | ASTBA093-19/ BOLD:ADI2769 | MN061567 | MN061522/ MN061523 |
| 8510-2 | <i>A. rupestris</i> | Cumbuca River | Cumbuca River | 13°00'36.9"S | 41°12'14.0"W | ASTBA094-19/ BOLD:ADI2769 | MN061568 | MN061518/ MN061519 |
| 8510-3 | <i>A. rupestris</i> | Cumbuca River | Cumbuca River | 13°00'36.9"S | 41°12'14.0"W | ASTBA095-19/ BOLD:ADI2769 | MN061569 | MN061520/ MN061521 |
| RPI7 | <i>A. aff rupestris</i> 1/ UFBA 06588 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA027-17/ BOLD:ACR6356 | KR007339 | - |
| RPI8 | <i>A. aff rupestris</i> 1 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA028-17/ BOLD:ACR6356 | KR007340 | - |
| RPII4 | <i>A. aff rupestris</i> 1 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA044-17/ BOLD:ACR6356 | KR007357 | MN061538/ MN061539 |
| RPII20 | <i>A. aff rupestris</i> 1 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA059-17/ BOLD:ACR6356 | KR007371 | - |
| RPI1 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA022-17/ BOLD:ACR6356 | KR007333 | KR007505/ KR007506 |
| RPI2 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA023-17/ BOLD:ACR6356 | KR007334 | MN061534/ MN061535 |
| RPI3 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA096-19/ BOLD:ACR6356 | KR007335 | KR007507/ KR007508 |
| RPI4 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA024-17/ BOLD:ACR6356 | KR007336 | KR007509/ KR007510 |
| RPI5 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA025-17/ BOLD:ACR6356 | KR007337 | KR007511/ KR007512 |
| RPI6 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA026-17/ BOLD:ACR6356 | KR007338 | KR007513/ KR007514 |
| RPI9 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA029-17/ BOLD:ACR6356 | KR007341 | KR007515/ KR007516 |
| RPI10 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA030-17/ BOLD:ACR6356 | KR007342 | KR007517/ KR007518 |
| RPI13 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA032-17/ BOLD:ACR6356 | KR007345 | KR007519/ KR007520 |
| RPI14 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA033-17/ BOLD:ACR6356 | KR007346 | KR007521/ KR007522 |
| RPI15 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA034-17/ BOLD:ACR6356 | KR007347 | KR007523/ KR007524 |
| RPI16 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA035-17/ BOLD:ACR6356 | KR007348 | KR007525/ KR007526 |
| RPI20 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA039-17/ BOLD:ACR6356 | KR007352 | KR007527/ KR007528 |
| RPI22 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA040-17/ BOLD:ACR6356 | KR007353 | KR007531/ KR007532 |
| RPII1 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA041-17/ BOLD:ACR6356 | KR007354 | KR007533/ KR007534 |
| RPII2 | <i>A. aff rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA042-17/ BOLD:ACR6356 | KR007355 | KR007535/ KR007536 |



TABLE S1 | (Continued)

| ID | Collection | | | | | Accession numbers | | |
|--------|--|------------------|----------------|--------------|--------------|------------------------------|------------------------|--------------------|
| | Sample | Species/Vouchers | Sub-basin | Site (river) | Latitude | Longitude | (COI/BIN) ¹ | Cytl ² |
| RPII3 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA043-17/ BOLD:ACR6356 | KR007356 | MN061536/ MN061537 |
| RPII6 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA046-17/ BOLD:ACR6356 | KR007359 | MN061540/ MN061541 |
| RPII7 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA047-17/ BOLD:ACR6356 | KR007360 | MN061542/ MN061543 |
| RPII10 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA049-17/ BOLD:ACR6356 | KR007363 | MN061544/ MN061545 |
| RPII15 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA054-17/ BOLD:ACR6356 | KR007368 | MN061549/ MN061549 |
| RPII16 | A. aff <i>rupestris</i> 2/ LISDEBE 7873 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA055-17/ BOLD:ACR6356 | MN061571 | MN061548/ MN061549 |
| RPII18 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA057-17/ BOLD:ACR6356 | KR007369 | KR007539/ KR007540 |
| RPII19 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA058-17/ BOLD:ACR6356 | KR007370 | KR007541/ KR007542 |
| RPII22 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA060-17/ BOLD:ACR6356 | KR007373 | KR007543/ KR007544 |
| RPII23 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA061-17/ BOLD:ACR6356 | KR007374 | KR007545/ KR007546 |
| RPII24 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA062-17/ BOLD:ACR6356 | KR007375 | KR007547/ KR007548 |
| RPII25 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA063-17/ BOLD:ACR6356 | KR007376 | MN061550/ MN061551 |
| RPII26 | A. aff <i>rupestris</i> 2 | Cumbuca River | Piabinha River | 12°59'35.9"S | 41°20'25.8"W | ASTBA064-17/ BOLD:ACR6356 | KR007377 | MN061552/ MN061553 |



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