**Table S1.** List of voucher specimens included in molecular analysis, locality data, GenBank accession numbers, and references. In bold, sequences produced by the present work. Other sequences were obtained from GenBank. Abbreviations of Brazilian states are: BA = Bahia, ES = Espírito Santo, PR = Paraná, RJ = Rio de Janeiro, SC = Santa Catarina, and SP = São Paulo.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Locality** | **Voucher specimen** | **GenBank accession number** | | | | | | **Reference** |
| **12S rRNA** | **16S rRNA** | **Cytb** | **RAG1(1)** | **RAG1(2)** | **tyr** |
| *Brachycephalus* *actaeus* | Municipality of São Francisco do Sul, SC | CFBH 44418 | MK697413 | MK697359 | MK697461 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *alipioi* | Municipality of Vargem Alta, ES | CFBH 11759 | MK697414 | MK697360 | MK697462 | HQ435718 | — | HQ435732 | **Clemente-Carvalho et al. (2011)** |
| *Brachycephalus albolineatus* | Morro Boa Vista, Jaraguá do Sul, SC | CFBH 41754 | — | MG889434 | — | — | — | — | **Monteiro et al. (2018)** |
| *Brachycephalus auroguttatus* | not given | not given | — | KX025367 | — | — | — | KX026215 | **Firkowski et al. (2016)** |
| *Brachycephalus boticario* | not given | not given | — | KX025374 | — | — | — | KX026223 | **Firkowski et al. (2016)** |
| *Brachycephalus bufonoides* | Serra Queimada trail, APA de Macaé de Cima, Lumiar, municipality of Nova Friburgo,RJ | ZUFRJ 15424 | — | MH259790 | — | — | — | — | **Folly et al. (2020)** |
| ZUFRJ 15426 | — | MH259791 | — | — | — | — | **Folly et al. (2020)** |
| ZUFRJ 15428 | — | MH259788 | — | — | — | — | **Folly et al. (2020)** |
| *Brachycephalus* *brunneus* | Pico Caratuva, municipality of Campina Grande do Sul, PR | CFBH 7905 | HQ435677 | HQ435691 | HQ435704 | HQ435719 | — | HQ435733 | **Clemente-Carvalho et al. (2011)** |
| *Brachycephalus* *clarissae*, new species | Theodoro de Oliveira, municipality of Nova Friburgo, RJ | MNRJ 56504 | MK697443 | MK697394 | MK697491 |  | **MT828479** | **MT828467** | **Condez et al. (2020); present study** |
| *Brachycephalus* *clarissae*, new species | Theodoro de Oliveira, municipality of Nova Friburgo, RJ | MNRJ 56516 |  | MK697395 |  |  | **MT828480** | **MT828468** | **Condez et al. (2020); present study** |
| *Brachycephalus* cf. *coloratus* | Morro do Canal, Piraquara, PR | not given | — | — | — | — | — | KX026118 | **Firkowski et al. (2016)** |
| *Brachycephalus* *crispus* | Parque Estadual da Serra do Mar, Bacia B, municipality of Cunha, SP | CFBH 27859 | MK697415 | MK697361 | MK697463 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *crispus* | Parque Estadual da Serra do Mar, Cedro Grande, municipality of Cunha, SP | CFBH 44400 | MK697416 | MK697362 | MK697464 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus curupira* | Serra do Salto, São José dos Pinhais, PR | not given | — | — | — | — | — | KX026228 | **Firkowski et al. (2016)** |
| *Brachycephalus* *didactylus* | Parque Estadual dos Três Picos, municipality of Cachoeiras de Macacu, RJ | MNRJ 54402 | MK697417 | — | — | — | **MT828473** | **MT828460** | **Condez et al. (2020); present study** |
| *Brachycephalus* *didactylus* | Reserva Ecológica de Guapiaçu, municipality of Cachoeiras de Macacu, RJ | MNRJ 68818 | MK697418 | MK697363 | MK697465 | — | **MT828474** | **MT828461** | **Condez et al. (2020); present study** |
| *Brachycephalus* *didactylus* | Municipality of Teresópolis, RJ | MNRJ 37327 | MK697419 | MK697364 | — | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *didactylus* | Vale da Revolta, municipality of Teresópolis, RJ | CFBH 11506 | — | MK697365 | — | JX267544 | — | — | **Canedo & Haddad (2012); Condez et al. (2020)** |
| *Brachycephalus* *ephippium* | Reserva Ecológica de Guapiaçu, municipality of Cachoeiras de Macacu, RJ | MNRJ 56517 | MK697420 | MK697366 | MK697466 | — | **MT828475** | **MT828462** | **Condez et al. (2020); present study** |
| *Brachycephalus* *ephippium* | Parque Nacional da Serra dos Órgaos, municipality of Teresópolis, RJ | CFBH 12355 | MK697421 | MK697367 | MK697467 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *ephippium* | Vale da Revolta, municipality of Teresópolis, RJ | MNRJ 50260 | MK697422 | MK697368 | MK697468 | — | — | **MT828463** | **Condez et al. (2020); present study** |
| *Brachycephalus* *ferruginus* | Parque Estadual do Marumbi, Pico Marumbi, municipality of Morretes, PR | CFBH 8025 | HQ435681 | HQ435695 | HQ435708 | HQ435723 | — | HQ435737 | **Clemente-Carvalho et al. (2011)** |
| *Brachycephalus fuscolineatus* | not given | not given | — | KX025341 | — | — | — | KX026189 | **Firkowski et al. (2016)** |
| *Brachycephalus* *garbeanus* | Municipality of Nova Friburgo, RJ | MNRJ 57293 | MK697423 | MK697369 | MK697469 | — | **MT828476** | **MT828464** | **Condez et al. (2020); present study** |
| *Brachycephalus* *garbeanus* | Alto de Macaé, municipality of Nova Friburgo, RJ | CFBH 12688 and CFBH 16800 | MK697424 | MK697370 | MK697470 | HQ435722 | — | HQ435736 | **Clemente-Carvalho et al. (2011); Condez et al. (2020)** |
| *Brachycephalus* *guarani* | Parque Estadual da Serra do Mar, Prumirim, municipality of Ubatuba, SP | CFBH 12997 | MK697425 | MK697371 | MK697471 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *hermogenesi* | Estação Biológica de Boracéia, municipality of Salesópolis, SP | TC 129 | — | MK697372 | — | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *hermogenesi* | Municipality of Paraibuna, SP | AAG-UFU177 | — | MK697373 | — | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *hermogenesi* | Parque Estadual da Serra do Mar, Picinguaba, municipality of Ubatuba, SP | CFBH 29793 | — | MK697374 | MK697472 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus hermogenesi* | Brazil: Piedade, SP | CFBH 12908 | HQ435682 | — | HQ435709 | HQ435724 | — | HQ435738 | **Clemente-Carvalho et al. (2011)** |
| *Brachycephalus* *ibitinga* | Parque das Neblinas, municipality of Mogi das Cruzes, SP | CFBH 14576 | MK697446 | MK697398 | MK697494 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *ibitinga* | Municipality of Itanhaém, SP | CFBH 19348 | MK697447 | MK697399 | MK697495 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *ibitinga* | Paranapiacaba, municipality of Santo André, SP | CFBH 14586 | MK697448 | MK697400 | MK697496 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *ibitinga* | Represa Billings, municipality of São Paulo, SP | CFBH 2978 | MK697449 | MK697401 | MK697497 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *ibitinga* | Parque Estadual Carlos Botelho, municipality of São Miguel Arcanjo, SP | CTMZ 2585 | MK697450 |  | MK697498 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *izecksohni* | Torre da Prata, municipality of Paranaguá, PR | CFBH 7374 | HQ435683 | HQ435696 | HQ435710 | HQ435725 | — | HQ435739 | **Clemente-Carvalho et al. (2011)** |
| *Brachycephalus leopardus* | not given | not given | — | KX025245 | — | — | — | KX026095 | **Firkowski et al. (2016)** |
| *Brachycephalus* *margaritatus* | Municipality of Paty do Alferes, RJ | MNRJ 76100 | MK697426 | MK697375 | MK697473 | — | **MT828477** | **MT828465** | **Condez et al. (2020); present study** |
| *Brachycephalus* *margaritatus* | Castelo Country Club, municipality of Petrópolis, RJ | MNRJ 73701 | — | MK697376 | MK697474 | — | **MT828478** | **MT828466** | **Condez et al. (2020); present study** |
| *Brachycephalus* *margaritatus* | Municipality of Petrópolis, RJ | CFBH 2420 | MK697427 | MK697377 | MK697475 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus mariaeterezae* | not given | not given | — | KX025350 | — | — | — | KX026199 | **Firkowski et al. (2016)** |
| *Brachycephalus mirissimus* | not given | not given | — | MH136570 | — | — | — | — | **Pie et al. (2018)** |
| *Brachycephalus* *nodoterga* | Parque Estadual de Ilhabela, municipality of Ilhabela, SP | CFBH 34963 | MK697428 | MK697378 | MK697476 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *nodoterga* | Brazil: Biritiba Mirim, SP | CFBH 6211 | HQ435697 | HQ435684 | HQ435711 | HQ435726 | — | HQ435740 | **Clemente-Carvalho et al. (2016)** |
| *Brachycephalus* *nodoterga* | Parque Estadual da Cantareira, municipality of Mairiporã, SP | CFBH 34912 | MK697429 | MK697379 | MK697477 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *nodoterga* | Estação Biológica de Boracéia, municipality of Salesópolis, SP | TC 128 | MK697430 | MK697380 | MK697478 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *nodoterga* | Municipality of Santana de Parnaíba, SP | CFBH 2226 | MK697431 | MK697381 | MK697479 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *olivaceus* | Castelo dos Bugres, municipality of Joinville, SC | CFBH 40440 and not given | MK697432 | MK697382 | MK697480 | — | — | KX026171 | **Condez et al. (2020); Firkowski et al. (2016)** |
| *Brachycephalus* *pernix* | Anhangava, municipality of Quatro Barrras, PR | CFBH 2597 | HQ435685 | HQ435698 | HQ435712 | HQ435727 | — | HQ435741 | **Clemente-Carvalho et al. (2011)** |
| *Brachycephalus* *pitanga* | Parque Estadual da Serra do Mar, municipality of São Luiz do Paraitinga, SP | CFBH 14868 and CFBH 16746 | MK697433 | MK697383 | MK697481 | HQ435728 | — | HQ435742 | **Clemente-Carvalho et al. (2011); Condez et al. (2020)** |
| *Brachycephalus* *pitanga* | Parque Estadual da Serra do Mar, municipality of Natividade da Serra, SP | CFBH 44411 | MK697434 | MK697384 | MK697482 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *pombali* | Pico da Igreja, municipality of Guaratuba, PR | CFBH 8043 | HQ435687 | HQ435700 | HQ435714 | HQ435729 | — | HQ435743 | **Clemente-Carvalho et al. (2011)** |
| *Brachycephalus* *pulex* | Serra Bonita, Municipality of Camacan, BA | TC 184 | — | MK697385 | — | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *quiririensis* | Municipality of Garuva, SC | CFBH 40406 and not given | MK697435 | MK697386 | MK697483 | — | — | KX026168 | **Firkowski et al. (2016); Condez et al. (2020)** |
| *Brachycephalus* *rotenbergae* | Joaquim Egídio, municipality of Campinas, SP | CFBH 2568 |  |  | MK697499 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *rotenbergae* | Municipality of Guaratinguetá, SP | CFBH 31982 | MK697451 | MK697402 | MK697500 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *rotenbergae* | Serra do Itapeti, municipality of Mogi das Cruzes, SP | CFBH 28662 | MK697452 | MK697403 | MK697501 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *rotenbergae* | Serra do Japi, municipality of Jundiaí, SP | CFBH 35773 | MK697453 | MK697404 | MK697502 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *rotenbergae* | Serra do Alambari, municipality of Resende, RJ | MNRJ 78465 | MK697454 | MK697405 | MK697503 | — | **MT828483** | — | **Condez et al. (2020); present study** |
| *Brachycephalus* *rotenbergae* | Municipality of Resende, RJ | MNRJ 73097 | MK697455 | — | MK697504 | — | **MT828484** | **MT828471** | **Condez et al. (2020); present study** |
| *Brachycephalus* *sulfuratus* | Parque Estadual da Ilha do Cardoso, municipality of Cananéia, SP | CFBH 39148 | MK697437 | MK697388 | MK697485 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *sulfuratus* | Parque Estadual do Rio Turvo, municipality of Barra do Turvo, SP | CTMZ 2970 | MK697438 | MK697389 | MK697486 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *sulfuratus* | Castelo dos Bugres, Municipality of Joinville, SC | CFBH 39146 | MK697439 | MK697390 | MK697487 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *sulfuratus* | Municipality of Quatro Barras, PR | CFBH 40436 | MK697440 | MK697391 | MK697488 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *sulfuratus* | Municipality of São Francisco do Sul, SC | CFBH 39141 | MK697441 | MK697392 | MK697489 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* *toby* | Parque Estadual da Serra do Mar, Pico do Corcovado, municipality of Ubatuba, SP | CFBH 44397 and CFBH 23002 | MK697436 | MK697387 | MK697484 | HQ435730 | — | HQ435744 | **Clemente-Carvalho et al. (2011); Condez et al. (2020)** |
| *Brachycephalus tridactylus* | Reserva Particular Salto Morato, Guaraqueçaba, PR and not given | not given | — | MG889431 | — | — | — | KX026237 | **Firkowski et al. (2016); Monteiro et al. (2018)** |
| *Brachycephalus verrucosus* | not given | not given | — | KX025234 | — | — | — | KX026170 | **Firkowski et al. (2016)** |
| *Brachycephalus* *vertebralis* | Parque Estadual da Serra do Mar, Picinguaba, municipality of Ubatuba, SP | CFBH 1389 and CFBH 7907 | MK697442 | MK697393 | MK697490 | HQ435731 | — | HQ435745 | **Clemente-Carvalho et al. (2011); Condez et al. (2020)** |
| *Brachycephalus* sp. 2 | Parque Estadual do Desengano, municipality of Santa Maria Madalena, RJ | CFBH 13724 | MK697444 | MK697396 | MK697492 | — | — | — | **Condez et al. (2020)** |
| MNRJ 42407 | — | **MT823010** | — | — | **MT828481** | **MT828469** | **present study** |
| MNRJ 52718 | — | **MT823011** | — | — | **MT828482** | **MT828470** | **present study** |
| *Brachycephalus* sp. 3 | Parque Estadual da Serra do Mar, municipality of Ubatuba, SP | CFBH 44409 | MK697445 | MK697397 | MK697493 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* sp. 6 | Estação Ecológica de Bananal, municipality of Bananal, SP | CFBH 44422 | MK697456 | MK697406 | MK697505 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* sp. 6 | Parque Estadual da Serra do Mar, Municipality of Cunha, SP | CFBH 44424 | MK697457 | MK697407 | MK697506 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* sp. 7 | Parque Estadual do Desengano, municipality of Santa Maria Madalena, RJ | CFBH 13722 | MK697458 | MK697408 | — | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* sp. 7 | Mendanha, municipality of Rio de Janeiro, RJ | MNRJ 58178 | — | MK697409 | — | — | **MT828485** | **MT828472** | **Condez et al. (2020)** |
| *Brachycephalus* sp. 8 | Ilha Grande, municipality of Angra dos Reis, RJ | CFBH 12907 | HQ435678 | HQ435692 | HQ435705 | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* sp. 8 | Municipality of Bananal, RJ | CTMZ 1522 | — | MK697410 | — | — | — | — | **Condez et al. (2020)** |
| *Brachycephalus* sp*.* 9 | Municipality of Corupá, SC | CFBH 42815 | MK697459 | MK697411 | MK697507 | — | — | — | **Condez et al. (2020)** |
| *Ischnocnema guentheri* | Brazil: Parque Nacional da Tijuca-RJ | CFBH 26993 | JX267331 | JX267501 and JX267502 | — | JX267611 | — | JX267746 | **Canedo & Haddad (2012)** |
| *Ischnocnema holti* | Brazil: Alto do Itatiaia, RJ | MNRJ 57645 | JX267306 | JX267306 | — | JX267617 | — | JX267754 | **Canedo & Haddad (2012)** |
| *Ischnocnema parva* | Brazil: Teresópolis, RJ | MNRJ 51356 | JX267438 | JX267379 | — | JX267649 | — | JX267787 | **Canedo & Haddad (2012)** |
| *Ischnocnema verrucosa* | Brazil: RPPN Serra Bonita, Camacan, BA | CFBH 23685 | JX267538 | JX267457 | — | JX267670 | — | JX267810 | **Canedo & Haddad (2012)** |
| *Oreobates cruralis* | Peru: Madre de Dios | KU 215462 | EF493662 | EF493662 | EU368881 | EU186743 | — | EU186764 | **Hedges et al. (2008)** |
| *Pristimantis ardilae* | Colombia: municipality of Toledo | MCNUPH163 | MN21541 | MN215428 | MN218379 | MN225946 | — | MN218364 | **Acevedo et al. (2020)** |

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