Recent dispersal and diversification within the clingfish genus *Acyrtus* (Actinopterygii: Gobiesocidae), with the description of a new western Atlantic species

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The genus *Acyrtus* (Gobiesocidae) is represented by four valid species distributed in the western Atlantic, and a recently described fifth species from the eastern Pacific. Here, we describe a new species endemic to Trindade Island, Brazil, and provide the first phylogenetic inference for the genus including all representatives. The new species can be distinguished from all its congeners by meristic and morphometric characters, as well as genetic differences. It presents low genetic diversity and, contrarily to other Trindade Island endemic fishes, shows no evidence of recent population growth. Our phylogeny reveals cryptic species and the paraphyletic nature of *Acyrtus*, which included *Arcos nudus* (western Atlantic) in a clade that separated from *Arcos erythrops* (tropical eastern Pacific) around 20 Mya. The three species found in the Brazilian Province, including one that remains undescribed, form a monophyletic clade which colonized the western South Atlantic around 2.6 Mya. Our study suggests that *Arcos nudus* should be placed in *Acyrtus*, and that the relationships among the closely-related Gobiesocidae genera *Acyrtus* (mostly from the Atlantic Ocean) and *Arcos* (from the Pacific Ocean) need further investigation.

**Keywords:** Brazilian Province, Endemism, Evolution, Phylogeny, Reef fish.
Phylogeny and new species of Acyrtus

O gênero Acyrtus (Gobiesocidae) é representado por quatro espécies válidas encontradas no Atlântico ocidental e uma recentemente descrita no Pacífico oriental. Aqui descrevemos uma nova espécie endêmica da Ilha da Trindade, Brasil, e apresentamos a primeira inferência filogenética para o gênero incluindo todos os representantes. A nova espécie pode ser distinguida de suas congêneres por caracteres merísticos e morfométricos, bem como por diferenças genéticas. A espécie apresenta baixa diversidade genética, entretanto, diferentemente de outras espécies endêmicas da Ilha da Trindade, não mostra evidência de expansão populacional recente. A filogenia obtida revelou a existência de espécies crípticas e a natureza parafilética de Acyrtus, o qual inclui Arcos nudus (do Atlântico ocidental), e que é separado de Arcos erythrops (do Pacífico tropical oriental) por cerca de 20 milhões de anos. As três espécies encontradas no Brasil, incluindo uma ainda não descrita, formam um clado monofilético que colonizou o Atlântico Sul ocidental há cerca de 2,6 milhões de anos. Nosso estudo sugere que Arcos nudus deva ser alocado no gênero Acyrtus, e que as relações entre os gêneros Acyrtus (em maioria do Oceano Atlântico) e Arcos (do Oceano Pacífico) precisam ser estudadas em mais detalhes.

Palavras-chave: Endemismo, Evolução, Filogenia, Peixes recifais, Província Brasileira.

INTRODUCTION

The cryptobenthic fish family Gobiesocidae (sensu Brandl et al., 2018), commonly known as clingfishes, comprises 190 recognized species (Fricke et al., 2022) distributed in freshwater, brackish, and coastal areas of the world’s tropical and subtropical regions (Briggs, 1955; Conway et al., 2017). Although occupying different environments and microhabitats (Gonçalves et al., 2002; Henriques et al., 2002), including a wide bathymetric range (Sparks, Gruber, 2012; Fricke et al., 2017), many species are morphologically similar and there is recognized uncertainty in their classification (Conway et al., 2020).

In this context, the use of molecular approaches combined with traditional taxonomy has provided a better understanding of evolutionary relationships (e.g., Henriques et al., 2002; Fricke et al., 2017; Wagner et al., 2019) and identification of cryptic lineages (e.g., Henriques et al., 2002; Craig, Randall, 2008; Conway et al., 2014; Wagner et al., 2019; Torres-Hernández et al., 2020). Though recent molecular studies have provided important progress in the knowledge of this family (Conway et al., 2017, 2020; Fricke et al., 2017), the evolutionary history of most species and the full extent of Gobiesocidae diversity remain unknown. Phylogenetic work including the New World Gobiesocinae suggested a recent diversification, the presence of several cryptic species and paraphyletic genera (Conway et al., 2014, 2017, 2020; Tavera et al., 2021), all of which we will explore in this study within the genus Acyrtus Schultz, 1944.

Acyrtus is represented by five valid species, Acyrtus artius Briggs, 1955, Acyrtus lanthanum Conway, Baldwin & White, 2014, Acyrtus pauciradiatus Sampaio, Anchieta,
Nunes & Mendes, 2004 and Acyrtus rubiginosus (Poey, 1868) are restricted to the western Atlantic, while Acyrtus arturo Tavera, Rojas-Vélez & Londoño-Cruz, 2021, was recently described from the eastern Pacific. These are small-bodied fishes (maximum size of less than 30 mm) with cryptobenthic habits, some presenting large secretory cells similar to those present in the venom glands of other teleost fishes (Conway et al., 2014). In the Atlantic, three species are found in the Caribbean and the fourth (A. pauciradiatus) is endemic to Fernando de Noronha Archipelago and Rocas Atoll, in Brazil. However, two other undescribed species have been found in restricted locations of the southwestern Atlantic, one on the Brazilian continental shelf and the other in the oceanic Trindade Island (Pinheiro et al., 2017).

Morphologically, Acyrtus is closely related to Rimicola Jordan & Evermann, 1896, a genus restricted to the eastern Pacific. However, a recent molecular phylogenetic study exploring Gobiesocidae has shown a close relationship between Acyrtus and Arcos Schultz, 1944 and, under low bootstrap values, has placed Arcos nudus (Linnaeus, 1758), from the western Atlantic, within the Acyrtus clade (Conway et al., 2014). This result agrees with the distribution of Arcos and Acyrtus genera since Arcos is mainly restricted to the eastern Pacific and Acyrtus to the western Atlantic. However, the recent description of A. arturo (Tavera et al., 2021) from Malpelo Island opens discussion about the evolutionary history of Acyrtus and Arcos. In addition, both genera share morphological characteristics that obscure their evolutionary proximity (e.g., Tavera et al., 2021). Species of both genera have previously been described as Gobiesox (Schultz, 1944; Conway et al., 2017), indicating that morphological similarities often lead to misidentifications (Conway et al., 2017). In this study, we provide the first phylogenetic inference for the genus Acyrtus containing all known species and including the undescribed species from Trindade Island as new, and an undescribed species from Brazilian coast. In addition, we analyzed the evolutionary history of the genus, and the phylogeographic and demographic history of the Brazilian species complex.

**MATERIAL AND METHODS**

**Morphological analysis.** The eight type specimens were collected with hand nets during a field expedition to Trindade Island in June 2009. Specimens were fixed in formalin 10% for 24 h, and then transferred to alcohol 70%. Counts were performed with a stereo microscope (Leica S9i, Amplification 6.1–55x) and X-rays obtained with a radiography system Faxitron LX60. Morphological characters were measured to the nearest 0.01 mm using an ocular micrometer mounted on a dissecting scope. Measurements and counts followed the methods presented in Briggs (1955), with the addition of predorsal and preanal lengths, which are the shortest distances between the tip of the upper lip and the dorsal and anal-fin origin, respectively (see Conway et al., 2014). Vertebral counts are presented as precaudal + caudal. The anterior-most vertebra with a haemal spine was counted as the first caudal vertebra, the urostylar complex the last. Following Smith-Vaniz (1971), the principal caudal ray counts included only those rays that articulate with the hypural plate, and are provided as upper+lower counts. Procurrent caudal rays are also provided as upper+lower counts. Measurements are expressed as a percentage of either standard length (SL) or head length (HL). Type
Phylogeny and new species of *Acyrtus*

Specimens were deposited in the fish collections of Universidade Federal do Espírito Santo, Vitória (CIUFES), California Academy of Sciences, San Francisco (CAS-ICH), Museu Nacional do Rio de Janeiro, Rio de Janeiro (MNRJ), Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP), Instituto de Biodiversidade e Sustentabilidade – NUPEM, Universidade Federal do Rio de Janeiro, Macaé (NPM), and Museu de Zoologia da Universidade Estadual de Campinas “Prof. Dr. Adão José Cardoso” (ZUEC). In the Description section, counts, measurements, and proportions of the holotype are followed by the range for paratypes inside parentheses. Teeth type and counts were obtained from a dissected specimen.

**Genetic data.** Cytochrome Oxidase I (COI) sequences of *Acyrtus* (*A. artius, A. arturo, A. lanthanum, A. rubiginosus, Acyrtus sp.n., and Acyrtus sp.*), *Arcos* (*A. erythrops* (Jordan & Gilbert, 1882) and *A. nudus*) and *Gobiesox* Lacepède, 1800 (*G. maenandricus* (Girard, 1858), *G. punctulatus* (Poey, 1876), and *G. rhesodon* Smith, 1881) were obtained from previous studies (Conway et al., 2014; Conway et al., 2017; Pinheiro et al., 2017; Tavera et al., 2021) and applied to phylogenetic and phylogeographic analyses. The DNA sequences from the Brazilian species *A. pauciradiatus* were extracted and amplified following protocols detailed in Weigt et al. (2012) for COI gene. Species of the families Gobiesocidae, Pseudochromidae, and Grammatidae were added as outgroups for the molecular-clock calibration. All sequences were aligned using ClustalW algorithm implemented in MEGA 7 (Kumar et al., 2016). Accession numbers of the sequences used are shown in Tab. S1.

**Phylogenetic analysis and molecular clock calibration.** Interspecific and intraspecific genetic divergences were calculated in MEGA 7 using Tamura-Nei model. Phylogenetic relationships among *Acyrtus* and *Arcos* were reconstructed by Bayesian Inference using Mr. Bayes 3.2.6 (Huelsenbeck, Ronquist, 1997). The analysis was performed for two parallel runs of 10 million generations, with four chains each and sampling trees every 1,000 generations. The burn-in value and the effective sample size (ESS) were assessed using Tracer 1.5 (Rambaut et al., 2018). All parameters exceed 200 in ESS values. The consensus tree was obtained from the maximum credibility clades with TreeAnnotator 1.7.5 (Drummond et al., 2012). The appropriate substitution model used was determined using PartitionFinder (Lanfear et al., 2017). We used SYM+I+G model for the first codon position, F81+I for the second position, and GTR+I+G for the third position. We used species of *Gobiesox* as outgroups based on Conway et al. (2017).

We estimated the divergence times among *Acyrtus* species using the Relaxed Clock Log Normal model and the Birth/Death prior implemented in BEAUTi & The BEAST 2.5.0 software (Bouckaert et al., 2014). Species of Gobiesocidae, Pseudochromidae, and Grammatidae family were incorporated in the alignment to implement a secondary calibration derived from the results of Near et al. (2013), which were also used by Conway et al. (2017) in the Gobiesocidae family phylogeny. We choose the nodes A (80.3 Ma), B (75.8 Ma), F (42.9 Ma), and G (23.1 Ma) from Conway et al. (2017) to represent the divergence of the last common ancestors between (A) Pseudochromidae + Grammatidae + Gobiesocidae, (B) Grammatidae + Gobiesocidae, (F) all Gobiesocidae species, and (G) *Acyrtus* + *Arcos* + *Gobiesox*. We constrained all node calibrations based on the topology obtained by Conway et al. (2017), which represents the most complete
Phylogeny obtained for the subfamily Gobiesocinae. The analysis was run in the Cipres Portal (http://www.phylo.org/) using 100 million generations and sampling every 3,000 generations. The effective sample size (ESS) and appropriate burn-in values were visualized in Tracer 1.5 (Rambaut et al., 2018). All parameters exceed 200 in ESS values. We used TreeAnnotator 1.4.3 (Drummond et al., 2012) to obtain the maximum clade credibility tree that was edited in FigTree (Rambaut, 2014) and Inkscape (Free Software Foundation, Boston, USA).

**Phylogeographic analysis and demography.** We calculated the genetic differentiation between Brazilian species (*A. pauciradiatus*, *Acyrtus* sp.n., and *Acyrtus* sp.) using the $F_{st}$ index (Wright, 1965) implemented in Arlequin 3.5.2.2 (Excoffier, Lischer, 2010). The COI haplotype network was constructed with the median-joining algorithm using PopArt (Bandelt et al., 1999). We performed demographic analysis only for *Acyrtus* sp.n. Haplotype and nucleotide diversity were estimated in DnaSP6 (Rozas et al., 2017) and neutrality tests were implemented in Arlequin 3.5.2.2. We used Tajima’s D and Fu’s F values to estimate possible events of population expansion. Finally, historical population dynamics were also evaluated using Bayesian Coalescent Skyline method implemented in Beast. We fixed the clock rate using the values obtained by the molecular clock analysis previously cited. The run comprised 10 million generations and samplings every 1,000 generations. We used Tracer 1.5 to check the parameters and to obtain the coalescent analysis output.

**RESULTS**

*Acyrtus simon* Gasparini & Pinheiro, new species

urn:lsid:zoobank.org:act:242B5ECF-E791-41DD-92E3-0BC8BA490910

(Fig. 1; Tab. 1)

*Arcos* sp. —Gasparini, Floeter, 2001:1646 [Trindade Island]. —Pereira-Filho et al., 2011:204 [Trindade Island]. —Simon et al., 2013:2123 [Trindade Island].

*Acyrtus* sp. —Macieira et al., 2015:390 [Trindade Island]. —Pinheiro et al., 2015:5 [Trindade Island]. —Pinheiro et al., 2017:83 [Trindade Island]. —Pinheiro et al., 2018:86 (Supplementary material) [Trindade Island]. —Guabiroba et al., 2020:701 [Martin Vaz Archipelago].

*Acyrtus* sp. nov. —Gasparini, 2017:78 [Trindade Island].

**Holotype.** CIUFES 2915, 26.41 mm SL, Brazil, Trindade Island, Calheta, 28 Jun 2009, H. T. Pinheiro & J. L. Gasparini (Fig. 1).

**Paratypes.** CAS-ICH 247280, 1, 22.21 mm SL; CIUFES 2914, 1, 26.00 mm SL; CIUFES 4448, 1 (dissected specimen), 32.23 mm SL; MNRJ 52794, 1, 24.71 mm SL; MZUSP 125855, 2, 21.81–25.44 mm SL; NPM 6839, 1, 25.79 mm SL; ZUEC 17336, 1, 30.60 mm SL, same data as for the holotype.
Phylogeny and new species of Acyrtus

FIGURE 1 | *Acyrtus simon*, holotype CIUFES 2915, 26.41 mm SL. A. Specimen alive, photo taken by J. L. Gasparini in June 2009; B–D. Specimen preserved, photo taken by R. M. Macieira on 31 October 2020. E. X-ray taken by M. M. Mincarone on 11 May 2022.
**Diagnosis.** *Acyrtus simon* differs from *A. artius* by having a deeper head depth (19–21% vs. 14–18% SL), a larger pelvic disc (36–39% vs. 29–36% SL), larger eye diameter (32–40% vs. 24–31% HL), and number of pectoral-fin rays (21–23 vs. 24–27) (Tabs. 1–2). *Acyrtus simon* can be distinguished from *A. lanthanum* by a deeper head depth (19–21% vs. 15–18% SL), larger eye diameter (32–40% vs. 24–31% HL), and number of pectoral-fin rays (21–23 vs. 24–25) (Tabs. 1–2). The new species differs from *A. rubiginosus* by a deeper head depth (19–21% vs. 13–16% SL), a larger pelvic disc (36–39% vs. 27–31% SL), wider pelvic disc (30–36% vs. 22–30% SL), larger eye diameter (32–40% vs. 23–28% HL), and number of pectoral-fin rays (21–23 vs. 24–27) (Tabs. 1–2). It differs from *A. pauciradiatus* by a larger pelvic disc (36–39% vs. 27–34% SL), shallower caudal peduncle (8–10% vs. 10–11% SL), longer caudal peduncle (10–17% vs. 5–8% SL), shorter anus–disc distance (6–12% vs. 12–13% SL), longer anus–anal fin distance (11–15% vs. 6–9% SL), longer snout length (20–33% vs. 9–15% HL), and narrower interorbital space (18–29% vs. 40–45% HL) (Tabs. 1–2). *Acyrtus simon* also differs from *Acyrtus arturo* by having shorter anus–disk distance (6–12 vs. 13–18% SL), longer anus–anal fin distance (11–15 vs. 4–9% SL), and longer snout length (20–33 vs. 10–15% HL) (Tabs. 1–2). It also differs from *Arcos nudus* [= *Acyrtus nudus*] by having a different number of pectoral-fin rays (21–23 vs. 23–25) (Tab. 1).

**Description.** Meristic and proportional measurements of the holotype and seven paratypes given in Tab. 1. Dorsal-fin rays 8 (8–9). Anal-fin rays 6 (6–8). Principal caudal-fin rays 5+5. Procurrent caudal-fin rays 6 (5–7) + 5 (5–7). Pectoral-fin rays 21 (21–23; one with 25). Pelvic-fin rays 1,4 (1, 4). Vertebrae 12 (11–13) + 17 (16–18). Body moderately depressed anteriorly, depth 4.9 (4.7–5.3) in SL. Head depressed, head width 2.4 (2.4–3.0) and head length 2.9 (2.1–2.9) in SL. Snout steep with a rounded outline, 3.1 (3.3–4.9) in head length. Posterior nostril surrounded by low fleshy rim and situated in front of anterior edge of eye; anterior nostril tubular, with a bifurcated cirri extending from posterior margin. Mouth terminal, upper lip broad, much wider in front of snout than on the sides. Upper jaw with 2+2 incisiform teeth anteriorly, followed by a single row of 10 small coniform teeth. Lower jaw with 2+2 incisiform teeth anteriorly, followed by single row of 6 coniform teeth. Diameter of eye 2.5 (2.5–3.1) and interorbital space 4.1 (3.4–5.4) in HL. Adhesive disc length 2.7 (2.5–2.7) and width 2.9 (2.8–3.3) in SL; 8 (7–9) transverse rows of papillae across width of disc region A; 10 (9–12) transverse rows of papillae across width of disc region B; 5–5 (3–5) longitudinal rows of papillae across width of disc region C. Pectoral length 4.8 (5.2–6.1) in SL. Pre-dorsal distance 1.4 (1.4–1.5). Dorsal length 5.7 (4.7–6.9). Caudal peduncle length 7.7 (5.9–10.4) and depth 11.0 (10.1–12.3) in SL.

**Color in alcohol.** Body overall pale, with orange blotches and bands on the dorsal side and on the head; fins hyaline; pupils hyaline with black margin; orange blotches on the iris (Fig. 1).

**Coloration in life.** Based on color photographs of live specimens (Figs. 1–2): body with variable red and white bands covered by small red spots; white bands might present red blotches; bands wider anteriorly and narrowing towards the caudal fin; pupil rounded and black, with thin white margin; white and red stripes and bands
**TABLE 1** | Proportional measurements and counts of type specimens of *Acyrtus simon*. H = Holotype.

|                  | CIUFES 2915 (H) | CAS 247280 | CIUFES 2914 | MNRJ 52794 | MZUSP 125855 | MZUSP 125855 | NUPEM 6839 | ZUEC 17336 |
|------------------|-----------------|------------|-------------|-------------|--------------|--------------|------------|-------------|
| Total length (in mm) | 32.19          | 26.94      | 30.91       | 30.26       | 32.94        | 26.50        | 31.68      | 36.38       |
| Standard length (SL, in mm) | 26.41          | 22.21      | 26.00       | 24.71       | 25.44        | 21.81        | 25.79      | 30.60       |
| **Measurements in % of SL** |                |            |             |             |              |              |            |             |
| Head length      | 33.7           | 36.5       | 34.9        | 35.0        | 47.3         | 40.9         | 36.5       | 34.2        |
| Head width       | 40.7           | 35.1       | 39.4        | 39.7        | 32.9         | 36.3         | 39.1       | 40.3        |
| Head depth       | 20.1           | 18.8       | 20.5        | 20.5        | 19.2         | 19.2         | 21.1       | 19.9        |
| Pelvic disc length | 37.2           | 37.9       | 38.1        | 38.9        | 39.2         | 36.2         | 37.1       | 37.8        |
| Pelvic disc width | 34.6           | 30.3       | 34.6        | 35.6        | 33.6         | 34.3         | 32.8       | 35.6        |
| Pre-dorsal distance | 68.7           | 64.1       | 68.5        | 67.7        | 65.4         | 65.5         | 67.7       | 65.7        |
| Pre-anal distance | 71.2           | 71.2       | 74.6        | 72.1        | 70.2         | 71.9         | 71.8       | 71.6        |
| dorsal length    | 17.3           | 20.7       | 14.3        | 16.5        | 20.9         | 16.4         | 18.4       | 15.7        |
| Pectoral length  | 20.6           | 16.3       | 19.0        | 15.4        | 16.2         | 18.4         | 16.9       | 15.9        |
| Caudal Peduncle length | 12.9          | 09.5       | 15.0        | 14.6        | 16.9         | 11.0         | 12.0       | 10.8        |
| Caudal peduncle depth | 09.0          | 09.6       | 08.1        | 09.8        | 08.2         | 09.6         | 09.4       | 08.6        |
| Anus-Disk distance | 07.1           | 09.5       | 09.1        | 08.7        | 05.8         | 12.4         | 07.3       | 11.2        |
| Anus-anal fin distance | 11.4          | 11.5       | 12.9        | 13.4        | 12.1         | 12.7         | 14.5       | 11.5        |
| Head length (HL, in mm) | 8.91           | 8.12       | 9.09        | 8.65        | 12.05        | 8.94         | 9.43       | 10.47       |
| **Measurements in % of HL** |                |            |             |             |              |              |            |             |
| Snout length     | 32.5           | 28.4       | 26.8        | 25.8        | 21.9         | 20.0         | 30.2       | 27.6        |
| Eye diameter     | 40.1           | 38.0       | 36.5        | 35.7        | 32.9         | 32.2         | 39.6       | 39.9        |
| Interorbital space | 24.2           | 26.1       | 18.2        | 18.3        | 20.4         | 19.2         | 29.2       | 24.0        |
| **Counts**       |                |            |             |             |              |              |            |             |
| Dorsal-fin rays  | 8              | 9          | 9           | 8           | 8            | 9            | 9          | 8           |
| Anal-fin rays    | 6              | 7          | 8           | 7           | 6            | 8            | 8          | 8           |
| Principal caudal-fin rays | 5+5           | 5+5        | 5+5         | 5+5         | 5+5          | 5+5          | 5+5        | 5+5         |
| Procurent caudal-fin rays | 6+5           | 7+6        | 7+5         | 6+5         | 6+5          | 5+5          | 7+7        | 6+5         |
| Pectoral-fin rays | 21             | 22         | 23          | 22          | 21           | 23           | 25         | 23          |
| Pelvic-fin rays  | 5              | 5          | 5           | 5           | 5            | 5            | 5          | 5           |
| Vertebrae        | 12+17          | 11+18      | 12+17       | 12+17       | 12+17        | 12+17        | 12+17      | 13+16       |
Thais L. Quintão, João Luiz Gasparini, Jean-Christophe Joyeux, Luiz A. Rocha and Hudson T. Pinheiro

**TABLE 2** Measurement comparisons among *Acyrtus* species. Data for *A. simon* and *A. pauciradiatus* were obtained in the present study. Data for *A. artius*, *A. lanthanum*, and *A. rubiginosus* were taken from Conway *et al.* (2014), and *A. arturo* from Tavera *et al.* (2021).

|                | *Acyrtus simon* | *A. pauciradiatus* | *A. artius* | *A. lanthanum* | *A. rubiginosus* | *A. arturo* |
|----------------|-----------------|-------------------|-------------|----------------|------------------|-------------|
| **Proportion in standard length** |                  |                   |             |                 |                  |             |
| Head length    | 34–47           | 40–44             | 44–47       | 39–43          | 32–39            | 41–43       |
| Head depth     | 19–21           | 18–2              | 14–18       | 15–18          | 13–16            | 19–27       |
| Pelvic disc length | 36–39          | 27–34             | 29–36       | 30–39          | 27–31            | 34–39       |
| Pelvic disc width | 30–36          | 32–36             | 29–34       | 31–38          | 22–30            | 31–32       |
| Pre-dorsal distance | 64–69          | 67–71             | 65–71       | 64–73          | 61–71            | 62–72       |
| Pre-anal distance | 70–75          | 69–76             | 67–78       | 71–77          | 66–80            | 70–76       |
| Caudal Peduncle length | 10–17          | 05–08             | 09–15       | 08–11          | 09–13            | 03–11       |
| Caudal peduncle depth | 08–10          | 10–11             | 07–10       | 09–14          | 07–09            | 07–09       |
| Anus-Disk distance | 06–12          | 12–13             | 08–15       | 04–08          | 12–14            | 13–18       |
| Anus-anal fin distance | 11–15          | 06–09             | 08–13       | 10–14          | 12–14            | 04–09       |
| **Proportion in head length** |                  |                   |             |                 |                  |             |
| Snout length   | 20–33           | 09–15             | 20–27       | 22–30          | 26–32            | 10–15       |
| Diameter eye   | 32–40           | 24–33             | 24–31       | 24–31          | 23–28            | 26–33       |
| Interorbital   | 18–29           | 40–45             | 14–21       | 15–21          | 18–25            | 18–25       |

Radiating outward from pupil across iris; iris also with thin white margin; first one-third of pectoral fin red, the posterior part hyaline; dorsal fin over red band red, and over white band hyaline with small red spots; caudal fin with variable white and red bands.

**Geographical distribution and habitat.** *Acyrtus simon* is only known from Trindade Island, situated 1,160 km from the Brazilian coast. There are unconfirmed records for its presence in the Martin Vaz Archipelago (Guabiroba *et al.*, 2020), which lies 40 km from Trindade. The species lives from intertidal habitat down to reefs 15 m depth (Fig. 2A). *Acyrtus simon* is commonly found taking shelter in cavities used by *Diadema antillarum* during the day (Fig. 2B), often sharing the protection from predators offered by spines with the Vitória–Trindade Chain (VTC) endemic *Hypleurochilus brasil* Pinheiro, Gasparini & Rangel, 2013, *Apogon americanus* Castelnau, 1855 and a number of others hosts.

**Etymology.** The specific name honors Thiony Emanuel Simon, our ichthyologist friend, who dedicated his career to study reef fishes, especially the fish biodiversity of the Vitória–Trindade Chain. To be treated as a noun in apposition.
Phylogeny and new species of *Acyrtus*

**Popular name.** Thiony’s clingfish; Peixe-ventosa de Thiony.

**Conservation status.** *Acyrtus simon* is endemic of Trindade Island. Endemic fishes from this island have not been assessed by the International Union for Conservation Nature (IUCN, 2021), with the exception of *Scartella poiti* Rangel, Gasparini & Guimarães, 2004; it is considered Vulnerable (VU) due the possibility of habitat degradation associated with its limited distributional range. The latest Brazilian Red List (Portaria MMA Nº 148, de 7 de junho de 2022) considers several Trindade Island endemic species as VU (i.e., *Halichoeres rubrovirens* Rocha, Pinheiro & Gasparini, 2010, *Malacocentrus brunoi* Guimarães, Nunan & Gasparini, 2010, *Stegastes trindadensis* Gasparini, Moura & Sazima, 1999, and *Sparisoma rocha* Pinheiro, Gasparini & Sazima, 2010).

**FIGURE 2** *Acyrtus simon* in the natural environment at Trindade Island. **A.** Photos taken during a night dive by J. L. Gasparini; and **B.** During the day by J-C. Joyeux.
2010, but not *S. poiti* nor *Elacatinus pridisi* Guimarães, Gasparini & Rocha, 2004), possibly for the same reasons, and in addition to the risks of fishing and ornamental trade. Therefore, *Acyrtus simon* is recommended to be categorized as VU according to the IUCN categories and criteria (IUCN Standards and Petitions Subcommittee, 2019).

**Phylogenetic analyses of Acyrtus.** COI sequences of 573 bp were obtained for 73 individuals of 20 species. Our analyses suggest the northwestern Atlantic as the center of the diversification of *Acyrtus*, and show that the genus is composed by at least nine species, forming a non-monophyletic group of three major clades (Fig. 3). One clade is composed by Caribbean and Brazilian species, another clade by the Caribbean *A. rubiginosus* lineage 1, *A. rubiginosus* lineage 2 and *Arcos nudus*, and a third solely composed by *A. arturo*, from the tropical eastern Pacific. Although three species (*A. artius*, *A. lanthanum* and *A. rubiginosus*) present a high distributional overlap along most of the Caribbean, our analysis revealed two cryptic species supported by high values of posterior probability, *Acyrtus* aff. *artius* from Tobago Island, and *Acyrtus* aff. *rubiginosus* from Belize (Fig. 3). Three species are endemic to the Brazilian Province and form a monophyletic group, which has *Acyrtus* aff. *artius* as the closest related species (Fig. 3). Brazilian species present small distributions: *Acyrtus simon* is restricted to Trindade Island, *A. pauciradiatus*, is restricted to Fernando de Noronha Archipelago and Rochas Atoll, and the undescribed species (*Acyrtus* sp., Fig. 3) is only known from the Salvador

![Bayesian phylogeny of Acyrtus based on the COI gene. Bayesian posterior probability values are shown and the biogeographic province of lineages presented in the right. SWA: southwestern Atlantic; NWA: northwestern Atlantic; EP: eastern Pacific.](image_url)
Phylogeny and new species of Acyrtus

region, on the northeastern Brazilian coast. In general, Caribbean species present higher interspecific divergence than Brazilian species (Tab. 3).

The estimated date for the most recent common ancestor of the Atlantic species and A. arturo (eastern Atlantic) is at least 15 Mya, and the origin of Arcos nudus and the Acyrtus rubiginosus clade was among the oldest diversification events of the genus within the Atlantic (Fig. 4; Tab. 4). Most of the diversification in Acyrtus is recent, occurring during the Pliocene and Pleistocene. The Brazilian clade is the youngest, diversifying around 2.55 Mya, and the divergence time between Acyrtus simon and Acyrtus sp. is around 1.7 Mya (Fig. 4). Divergences were higher among Caribbean species than among Brazilian species.

### TABLE 3

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|----|
| 1. Acy sim | 0.11 |   |   |   |   |   |   |   |   |    |    |    |    |
| 2. Acy sp  | 1.25 | 0.23 |   |   |   |   |   |   |   |    |    |    |    |
| 3. Acy pau  | 1.85 | 1.93 | 0.09 |   |   |   |   |   |   |    |    |    |    |
| 4. Acy aff arti | 5.71 | 6.60 | 6.10 | – |   |   |   |   |   |    |    |    |    |
| 5. Acy arti | 6.60 | 7.61 | 7.85 | 7.08 | 0.45 |   |   |   |   |    |    |    |    |
| 6. Acy lan | 7.62 | 10.31 | 10.46 | 11.16 | 10.09 | 0.40 |   |   |   |    |    |    |    |
| 7. Acy artu | 19.36 | 19.63 | 19.55 | 19.30 | 17.59 | 20.51 | 0.00 |   |   |    |    |    |    |
| 8. Acy rub 1 | 19.42 | 18.50 | 18.84 | 17.92 | 18.08 | 20.03 | 20.63 | 0.96 |   |    |    |    |    |
| 9. Acy rub 2 | 20 | 19.68 | 20.08 | 19.80 | 19.06 | 19.46 | 20.91 | 5.81 | 0.12 |   |    |    |    |
| 10. Arc nud | 19.94 | 20.57 | 19.64 | 21.38 | 20.52 | 19.38 | 21.33 | 20.89 | 19.18 | – |    |    |    |
| 11. Arc ery | 31.60 | 31.63 | 31.12 | 30.60 | 30.82 | 30.46 | 31.47 | 32.00 | 33.79 | 31.33 | – |    |    |
| 12. Gob pun | 26.28 | 27.41 | 26.07 | 27.22 | 25.77 | 25.27 | 25.33 | 28.31 | 29.57 | 29.24 | 36.53 | – |    |
| 13. Gob mae | 26.97 | 26.53 | 27.68 | 28.53 | 30.53 | 27.69 | 27.95 | 26.98 | 27.19 | 27.88 | 36.10 | 27.55 | – |
| 14. Gob rhe | 28.35 | 28.62 | 29.89 | 29.22 | 28.93 | 28.41 | 28.64 | 28.42 | 27.86 | 27.56 | 37.69 | 29.57 | 18.55 |
Evolutionary history of *Acyrtus simon*. The close relationship between *Acyrtus simon*, from Trindade Island, and *Acyrtus* sp. from the Brazilian coast was also revealed in the haplotype network (Fig. 5) and through smaller $F_{ST}$ values than *Acyrtus pauciradiatus* (Tab. 5). *Acyrtus simon*, represented by only three haplotypes (Fig. 5), presented low haplotype and nucleotide diversity ($Hd = 0.362$; $\pi = 0.001$). Neutrality tests presented negative values (Tab. 6), though the Skyline plot did not present evidence of recent population growth in Trindade Island (Fig. 6) due to the low number of haplotypes.
**TABLE 4** | Divergence times (Mya) among *Acyrtus* species and *Arcos nudus*, and the 95% highest posterior density.

| Node                                               | Divergence times | 95% HPD     |
|----------------------------------------------------|------------------|-------------|
| Node *Acyrtus* + *Arcos nudus*                     | 15.5             | 10.1–20.9   |
| *Arcos nudus* / *Acyrtus rubiginosus* clade         | 8.65             | 3.6–13.7    |
| Northwestern AT/AT southwestern AT                 | 3.9              | 1.4–6.4     |
| Brazilian clade                                    | 2.55             | 0.8–4.3     |
| *Acyrtus simon* / *Acyrtus* sp.                    | 1.7              | 0.4–3.0     |
| *Acyrtus simon*                                    | 0.95             | 0.2–1.7     |

**FIGURE 5** | Haplotype network of *Acyrtus artius* and the representatives of *Acyrtus* in the southwestern Atlantic. FN = Fernando de Noronha.
**TABLE 5** | $F_{st}$ index among *Acyrtus* in the Southwestern Atlantic. All values were significant (p<0.05).

|                | 1       | 2       | 3       |
|----------------|---------|---------|---------|
| 1. *Acyrtus simon* (Trindade Island) | –       |         |         |
| 2. *Acyrtus* sp. (Brazilian coast)  | 0.89    | –       |         |
| 3. *Acyrtus pauciradiatus* (FN Archipelago) | 0.94    | 0.92    | –       |

**TABLE 6** | Diversity and neutrality indexes calculated for *Acyrtus simon*, Trindade Island. h = haplotypes number; Hd = haplotype diversity; SD Hd = standard deviation of Hd; π = nucleotide diversity; SD π = standard deviation of π.

| h   | Hd    | SD Hd  | Pi    | SD Pi  | Fu’s F | Tajima’s D |
|-----|-------|--------|-------|--------|--------|------------|
| 3   | 0.362 | 0.145  | 0.00110 | 0.00048 | -0.0063 | -0.9481 |

**FIGURE 6** | Bayesian skyline plot showing the effective population size fluctuation of *Acyrtus simon* through time (Mya) (black line: median estimation; purple: confidence interval).
DISCUSSION

Our study presents the first phylogeny of the genus *Acyrtus* including all known representatives, revealing: 1) insights on the evolutionary history of the genus; 2) the current paraphyletic status of the genus; 3) the Brazilian Province species complex as a monophyletic group; 4) and the presence of undescribed and cryptic species. Although our phylogenetic inference is based on a single DNA marker, our conclusions are based on high statistical support.

The absence of reliable fossil data for the family Gobiesocidae (but see Schwarzhans et al., 2017) constitutes as one of the main barriers to the understanding of its evolutionary history. The secondary calibration from the divergence times obtained by Near et al. (2013), and used by Conway et al. (2017), was used here to study the genus *Acyrtus*. Near et al. (2013) estimated the origin of the family Gobiesocidae in the Eocene, around 42.9 Mya, which is consistent with the emergence of most reef fish families, between 66 and 34 Mya (Bellwood, 2015). Around 39 Mya (Conway et al., 2017), shortly after the origin of the family Gobiesocidae, its New World lineage (subfamily Gobiesocinae) was formed during a period marked by great diversification in the Tethys Sea (Renema et al., 2008). It is possible that earlier Gobiesocidae lineages originated in the Tethys Sea and then colonized both the Atlantic/eastern Pacific and the Indian-western Pacific oceans (Floeter et al., 2008). However, earlier Gobiesocidae lineages could also have had their origins in any of the oceans, using the Tethys as a passage. For instance, Gobiosomatini (Gobiidae) (Thacker, 2015), also endemic of the new world, originated in a period similar to Gobiesocinae, likely via dispersal from the western Indian Ocean through the Tethys passage (Thacker, 2015). While little can be concluded about the center of origin of Gobiesocidae, it seems that the Tethys Sea was important for dispersal and early diversification in the family.

After the rise of the Gobiesocinae, *Acyrtus* lineages and *Arcos erythrops* diverged much earlier than the closure of the Isthmus of Panama, around ~21 Mya. Therefore, this event did not influence the divergence between Gobiesocinae genera as commonly seen in other groups (Lessios, 2008). Tavera et al. (2021) found a similar divergence time for *Arcos erythrops*, although the placement of this species did not evidence the monophyly of *Arcos* and *Acyrtus*. Despite this, our results support the topology obtained by Conway et al. (2020) based on seven different genes. Nevertheless, further investigation of the *Arcos* phylogeny with broader taxon sampling is needed.

Our results also show the emergence of the western Atlantic *Acyrtus* at least 15 Mya (the age of the last common ancestor of *Acyrtus + Arcos nudus*) in a period of origin and diversification of most reef fish genera (Bellwood et al., 2015). Moreover, the origin of *Acyrtus* coincides with the emergence of the Amazon barrier, which could have prevented an earlier dispersal from the Caribbean to the Brazilian Province. It could explain the latter diversification of *Acyrtus* in the history of this genus (~3.9 Mya). In this case, the glacioeustatic sea-level changes of the Pleistocene/Pliocene could have contributed to the connectivity between regions, and to the crossing of the biogeographic barrier, as suggested for many other reef fishes (Rocha, 2003).

The diversification of the Brazilian complex of species started during the Pleistocene and these three species present restricted distributions: two are endemic to oceanic islands, and a third is only known from a narrow geographic range of the Brazilian
northeastern coast. There are two hypotheses for speciation and colonization routes. The first involves the colonization of the Fernando de Noronha archipelago, with posterior colonization of the Brazilian coast, which recently colonized Trindade Island. This hypothesis is corroborated by the fact that a few Caribbean species are also found in Fernando de Noronha but not along the Brazilian coast (Rocha, 2003). This archipelago could be a gateway for Caribbean species to colonize Brazilian waters. A second hypothesis would involve the colonization of the Brazilian coast first, with a subsequent and earlier colonization of Fernando de Noronha, and a more recent colonization of Trindade also from the coast. Some reef fish lineages were more widely distributed along the southwestern Atlantic in the past, persisting as relicts in restricted locations (Rocha et al., 2010; Pinheiro et al., 2017). The exposure of seamounts during low sea-levels could have favored the colonization of the Vitória–Trindade Chain (Macieira et al., 2015; Pinheiro et al., 2017).

Even though we observed low genetic diversity in our results for *A. simon*, there was no sign of population bottlenecks or expansion in the neutrality test, differently from other VTC endemics (Pinheiro et al., 2017). Differently from species with wider distribution (Pinheiro et al., 2017), the restriction of *Acyrtus* to shallow reefs and its absence on the VTC seamounts may have constrained its connectivity between the coast and the islands and limited the input of new haplotypes. Recent population expansions are seen in many species along the western Atlantic (da Silva et al., 2015; Liedke et al., 2020) that seem to be related to a ~90% increase in reef area caused by the rise in sea-level following the last glacial maximum (Ludt, Rocha, 2015).

Both Conway et al. (2014) and our study show three undescribed Caribbean *Acyrtus* species, one of them closely related to *Acyrtus artius* and the other two to *A. rubiginosus*. The existence of many hidden lineages in the same genus may be attributed to the species small size, cryptic behavior, and a low morphological divergence. The latter, in particular, is commonly related to recent speciation and/or stabilizing selection on the ancestral phenotype (Milá et al., 2017). A similar pattern is found for *Gouania* (Gobiesocidae) in the Mediterranean Sea, where recent diversification and cryptic lineages were recognized by Wagner et al. (2019). These results evidence that future molecular studies for Gobiesocidae should lead to the discovery of many cryptic species. The most likely cause for the high diversification in this family (even within provinces) is related to their weak dispersal potential, small size, sedentary habit, and demersal eggs (Pires, Gibran, 2011). These life-history characteristics are among the most important drivers of speciation in the Brazilian Province (Pinheiro et al., 2018; Mazzei et al., 2021; Simon et al., 2021), and other taxonomic groups that share similar traits (e.g., Labrisomidae and Gobiidae) also show strong genetic structure and cryptic speciation within the Great Caribbean (Baldwin et al., 2011; Victor, 2014). Additionally, the distribution of the closely-related Brazilian *Acyrtus* species in different environments (mainland coast and oceanic islands) and localities with distinct levels of isolation suggests that ecological and peripatric speciation processes might be important drivers of diversification in Gobiesocidae (Rocha et al., 2005; Pinheiro et al., 2017; Simon et al., 2021).

Our phylogenetic inference discloses the paraphyletic nature of *Acyrtus*, which includes *Arcos nudus* in its clade. The first *Acyrtus* species (*A. rubiginosus*) was described in 1868, originally assigned to the genus *Sicyases*, a valid genus described based on *Sicyases*
Phylogeny and new species of Acyrtus

The genera *Acyrtus* and *Arcos* were described in the same article (Schultz, 1944), based on *Acyrtus rubiginosus* and *Arcos erythrops*, respectively. *Arcos nudus* was originally described by Linnaeus (1758) as *Cyclopterus*, a valid and now monotypic genus described based on *Cyclopterus lumpus* Linnaeus, 1758 (Cyclopteridae or lumpfishes). It was later reassigned as *Gobiesox nudus* by Briggs (1955), and afterwards as *Arcos nudus* by Fernholm, Wheeler (1983). All other *Arcos* species are from the tropical eastern Pacific and were originally described as *Gobiesox*, a valid genus described based on *Gobiesox cephalus* Lacépède, 1800.

Our phylogenetic analyses indicate that *Arcos nudus* should be reassigned to *Acyrtus*. This species shares several distinguishing morphological characters with other *Acyrtus* species, including large secretory cells present inside the groove present in the subopercular spine (Conway *et al.*, 2014). Therefore, according to our data, the genus *Arcos* seems to be exclusive to the eastern Pacific, while most *Acyrtus* are found in the western Atlantic. Additional studies including more eastern Pacific Gobiesocidae species are necessary to better assess the status of *Acyrtus arturo*, from Malpelo Island (Tavera *et al.*, 2021), and *Arcos nudus*, from the Atlantic, which could belong to other clades. Alternatively, a more complete phylogenetic analysis including all *Arcos* species could also show clades uniting *Acyrtus* and *Arcos erythrops*, suggesting the unification of *Acyrtus* and *Arcos* in a single genus. Finally, our results highlight that a broad sampling in Gobiesocidae family will bring important insights about evolutionary patterns of cryptobenthic fishes.

**Comparative material examined.** All from Brazil. *Acyrtops beryllinus*: CIUFES 2930, 3, 12.36–16.52 mm SL. CIUFES 130851, 1, 25.12 mm SL; *Acyrtus pauciradiatus*: CIUFES 2475, 1, 11.08 mm SL. CIUFES 2481, 2, 9.36–11.24 mm SL. CIUFES 2503, 1, 19.02 mm SL. CIUFES 2548, 1, 10.03 mm SL. *Gobiesox barbatulus*: CIUFES 307, 6, 20.26–47.64 mm SL. CIUFES 1368, 14, 20.26–47.64 mm SL. CIUFES 2923, 3, 34.30–49.72 mm SL. CIUFES 2883, 6, 13.14–30.47 mm SL. *Tomicodon australis*: CIUFES 130269, 2, 19.78–25.31 mm SL. CIUFES 130294, 2, 14.11–19.12 mm SL. CIUFES 130712, 1, 15.42 mm SL. CIUFES 130853, 13, 20.36–31.21 mm SL.

**ACKNOWLEDGMENTS**

We thank the Brazilian Navy for support during scientific expeditions to Trindade Island. We also thank Raphael M. Macieira, C. L. S. Sampaio, R. G. Santos for support in the field and with samples, Fabio Di Dario, Kevin W. Conway and Sergio R. Floeter for providing valuable literature. TLQ is supported by a FAPES doctoral fellowship (Fundação de Amparo à Pesquisa e Inovação do Espírito Santo), JLG by a CAPES doctoral fellowship (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and HTP by a FAPESP JP fellowship (Fundação de Amparo à Pesquisa do Estado de São Paulo; 2019/24215–2; 2021/07039–6). LAR and HTP are funded by the Hope for Reefs initiative of the California Academy of Sciences. We thank Michael M. Mincarone, Raphael M. Macieira, and an anonymous reviewer for their insightful and constructive comments on the various versions of our manuscript.
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ETHICAL STATEMENT

Fish collection was authorized by the Brazilian Environmental Agency, Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO 12786–1 and 20880–2), and was in accordance with the ethical principles for animal experimentation, approved by the Ethics Committee for the Use of Animals of the Universidade Federal do Espírito Santo (CEUA-UFES 017–2009).

COMPETING INTERESTS

The authors declare no competing interests.

HOW TO CITE THIS ARTICLE

• Quintão TL, Gasparini JL, Joyeux J-C, Rocha LA, Pinheiro HT. Recent dispersal and diversification within the clingfish genus Acyrtus (Actinopterygi: Gobiesocidae), with the description of a new western Atlantic species. Neotrop Ichthyol. 2022; 20(3):e210137. https://doi.org/10.1590/1982-0224-2021-0137