

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 erythroptus* (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.

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O gênero *Acyrthus* (Gobiesocidae) é representado por quatro espécies válidas encontradas no Atlântico ocidental e uma recentemente descrita do 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 *Acyrthus*, o qual inclui *Arcos nudus* (do Atlântico ocidental), e que é separado de *Arcos erythropus* (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 *Acyrthus*, e que as relações entre os gêneros *Acyrthus* (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 *Acyrthus* Schultz, 1944.

Acyrthus is represented by five valid species, *Acyrthus artius* Briggs, 1955, *Acyrthus lanthanum* Conway, Baldwin & White, 2014, *Acyrthus 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

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. maeandricus* (Girard, 1858), *G. punctulatus* (Poey, 1876), and *G. rhessodon* 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-92E3-0BC8BA496910

(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.

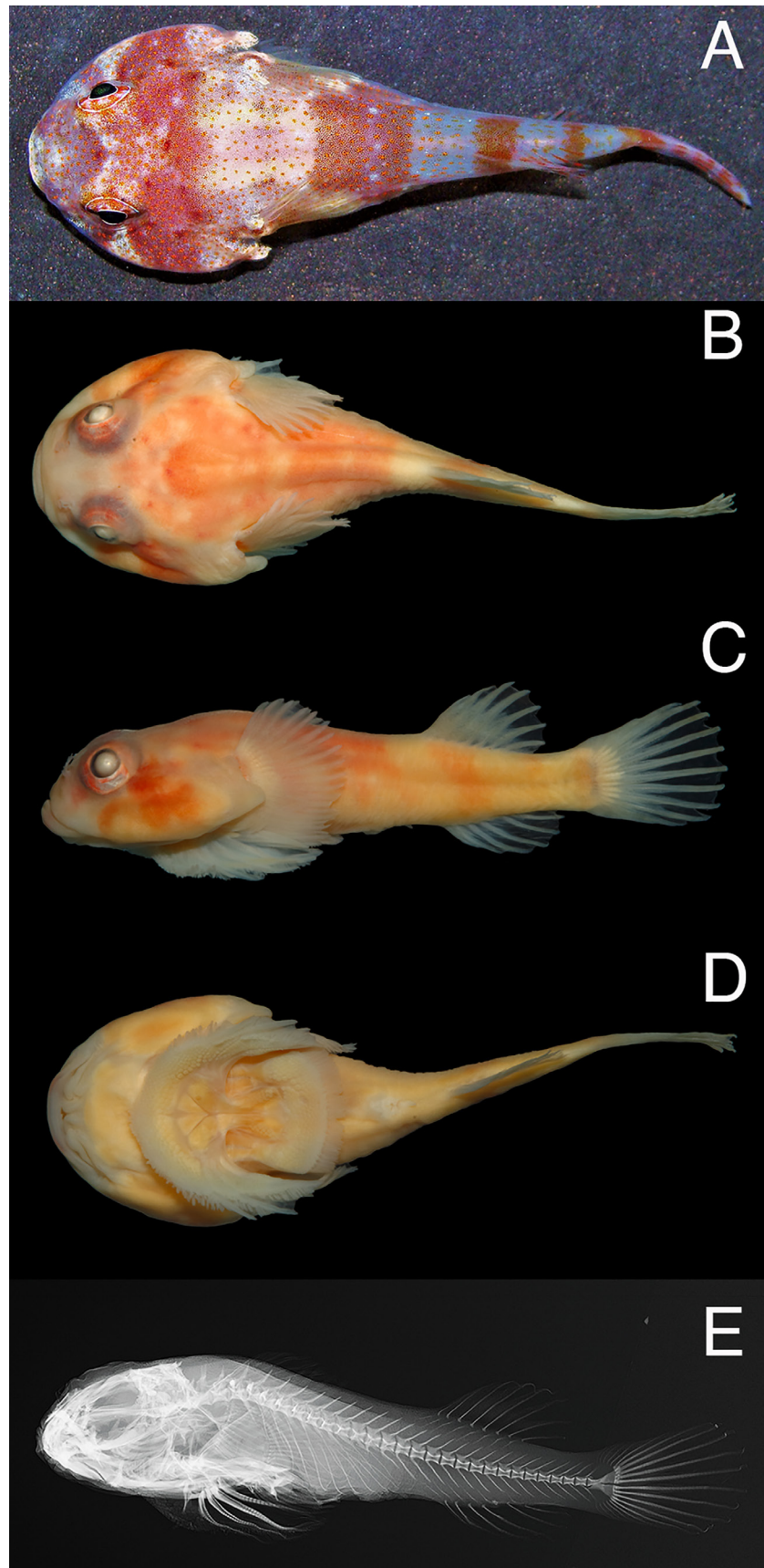


FIGURE 1 | *Acyrtus simon*, holotype CIUFES 2915, 26.41 mm SL. **A.** Specimen alive, photo taken by J. L. Gasparini in June 2009; **B–D.** Specimen preserved, photo taken by R. M. Macieira on 31 October 2020. **E.** X-ray taken by M. M. Mincarone on 11 May 2022.

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 I,4 (I, 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
Procurrent 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

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).

	<i>Acyrtus simon</i>	<i>A. pauciradiatus</i>	<i>A. artius</i>	<i>A. lanthanum</i>	<i>A. rubiginosus</i>	<i>A. arturo</i>
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.

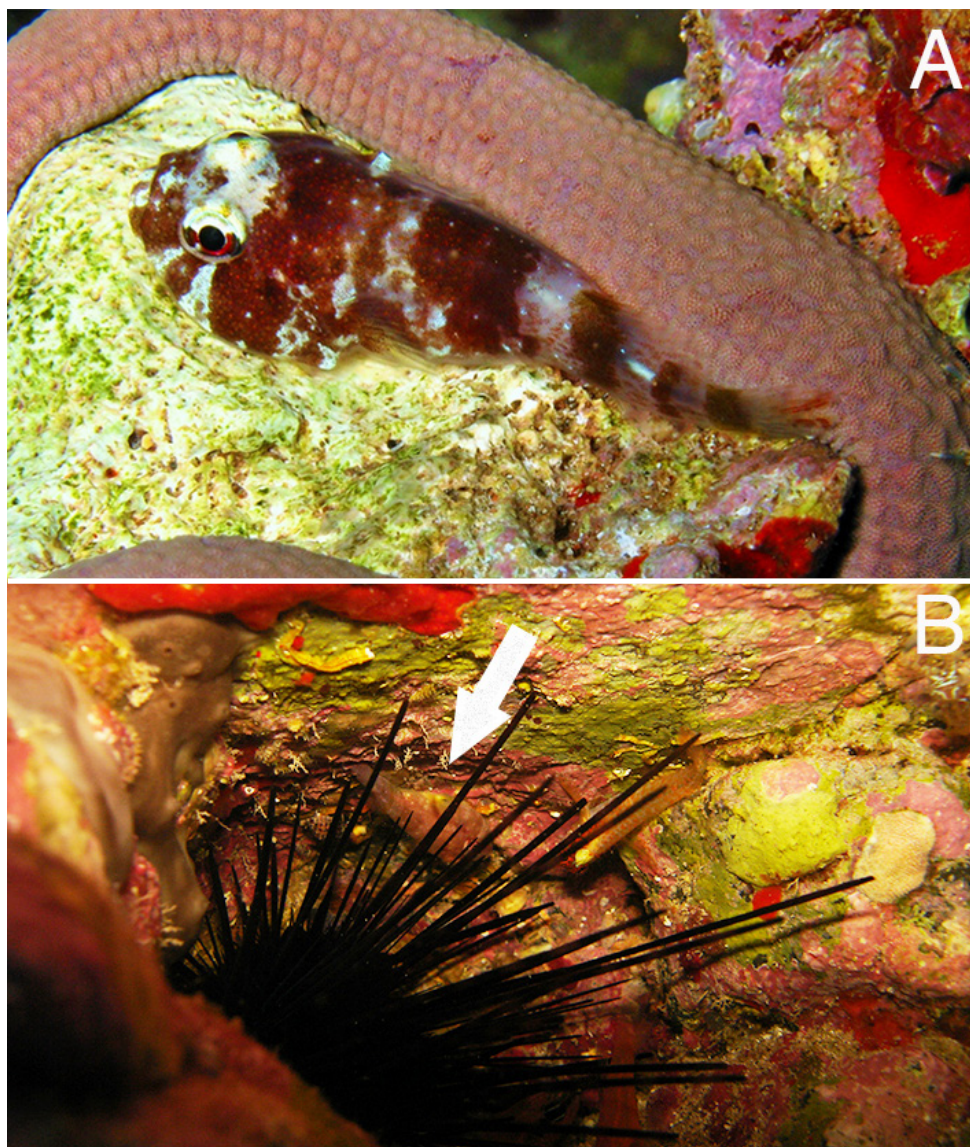


FIGURE 2 | *Acyrtus simon* in the natural environment at Trindade Island. **A.** Photos taken during a night dive by J. L. Gasparini; and **B.** During the day by J-C. Joyeux.

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 N° 148, de 7 de junho de 2022) considers several Trindade Island endemic species as VU (*i.e.*, *Halichoeres rubrovirens* Rocha, Pinheiro & Gasparini, 2010, *Malacotenus brunoi* Guimarães, Nunan & Gasparini, 2010, *Stegastes trinidadensis* Gasparini, Moura & Sazima, 1999, and *Sparisoma rocha* Pinheiro, Gasparini & Sazima,

TABLE 3 | Interspecific and intraspecific (blue boxes) divergence among species of *Acyrtus*, *Arcos*, and *Gobiesox* (percentage values – %). *Acy sim*: *Acyrtus simon*; *Acy sp*: *Acyrtus sp*; *Acy pau*: *Acyrtus pauciradiatus*; *Acy aff arti*: *Acyrtus aff. artius*; *Acy arti*: *Acyrtus artius*; *Acy lan*: *Acyrtus lanthanum*; *Acy rub 1*: *Acyrtus rubiginosus* lineage 1; *Acy rub 2*: *Acyrtus rubiginosus* lineage 2; *Acy artu*: *Acyrtus arturo*; *Arc nud*: *Arcos nudus*; *Arc ery*: *Arcos erythroptus*; *Gob pun*: *Gobiesox punctulatus*; *Gob rhe*: *Gobiesox rhessodon*; *Gob mae*: *Gobiesox maeandricus*.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. <i>Acy sim</i>	0.11												
2. <i>Acy sp</i>	1.25	0.23											
3. <i>Acy pau</i>	1.85	1.93	0.09										
4. <i>Acy aff arti</i>	5.71	6.60	6.10	–									
5. <i>Acy arti</i>	6.60	7.61	7.85	7.08	0.45								
6. <i>Acy lan</i>	7.62	10.31	10.46	11.16	10.09	0.40							
7. <i>Acy artu</i>	19.36	19.63	19.55	19.30	17.59	20.51	0.00						
8. <i>Acy rub 1</i>	19.42	18.50	18.84	17.92	18.08	20.03	20.63	0.96					
9. <i>Acy rub 2</i>	20	19.68	20.08	19.80	19.06	19.46	20.91	5.81	0.12				
10. <i>Arc nud</i>	19.94	20.57	19.64	21.38	20.52	19.38	21.33	20.89	19.18	–			
11. <i>Arc ery</i>	31.80	31.63	31.12	30.60	30.82	30.46	31.47	32.00	33.79	31.33	–		
12. <i>Gob pun</i>	26.28	27.41	26.07	27.22	25.77	25.27	25.33	28.31	29.57	29.24	36.53	–	
13. <i>Gob mae</i>	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. <i>Gob rhe</i>	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

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.

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 ($H_d = 0.362$; $\pi = 0.001$). 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.

FIGURE 4 | Bayesian estimates of divergence time based on the mitochondrial COI gene. Posterior probability values reached 1–0.99 for all the main nodes (represented by the orange circle). The horizontal purple bars indicate 95% credibility intervals of node age estimation. The calibration nodes 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* (see Conway *et al.*, 2017).

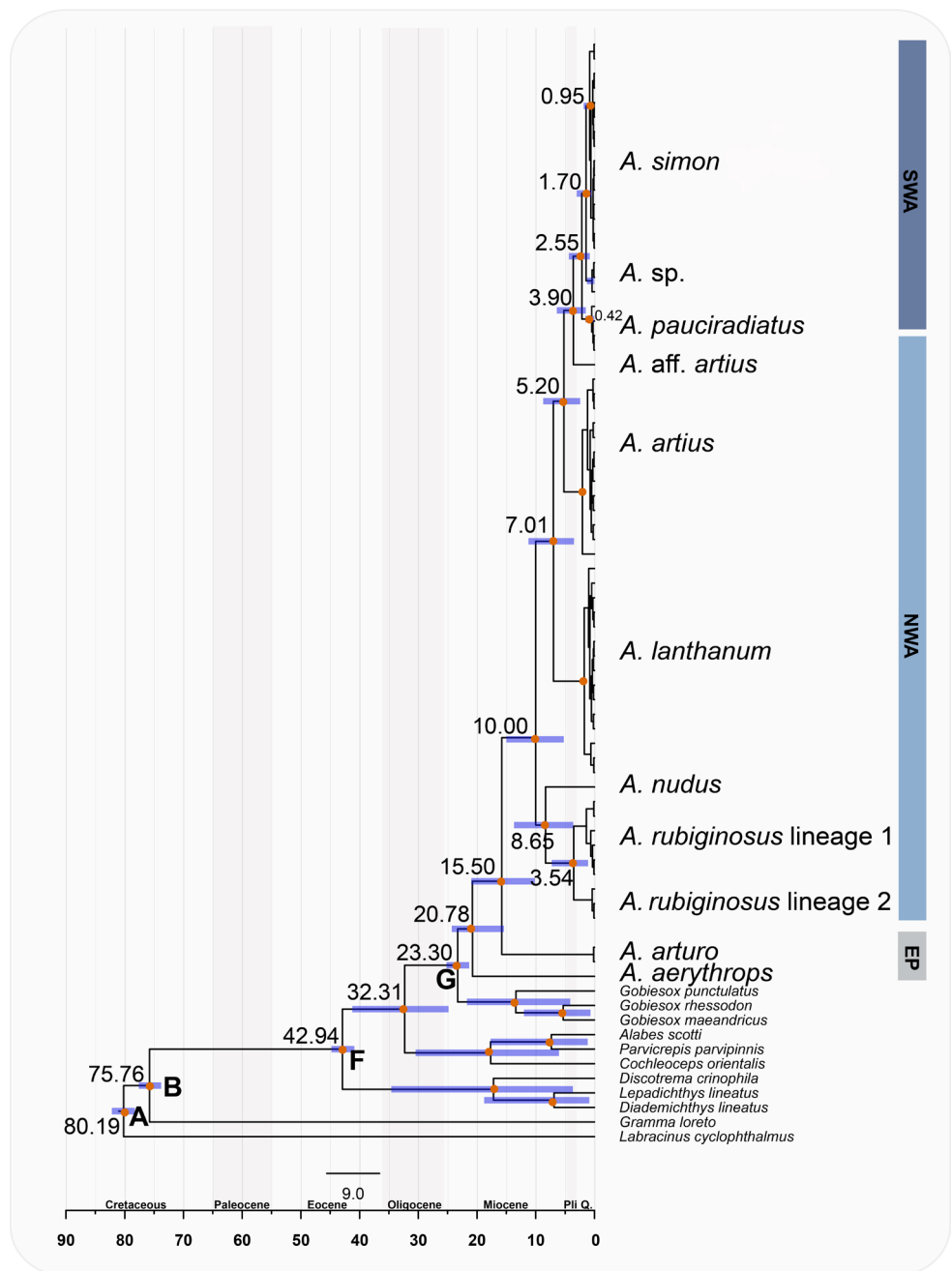


TABLE 5 | F_{ST} index among *Acyrtus* in the Southwestern Atlantic. All values were significant ($p < 0.05$).

	1	2	3
1. <i>Acyrtus simon</i> (Trindade Island)	–		
2. <i>Acyrtus</i> sp. (Brazilian coast)	0.89	–	
3. <i>Acyrtus pauciradiatus</i> (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

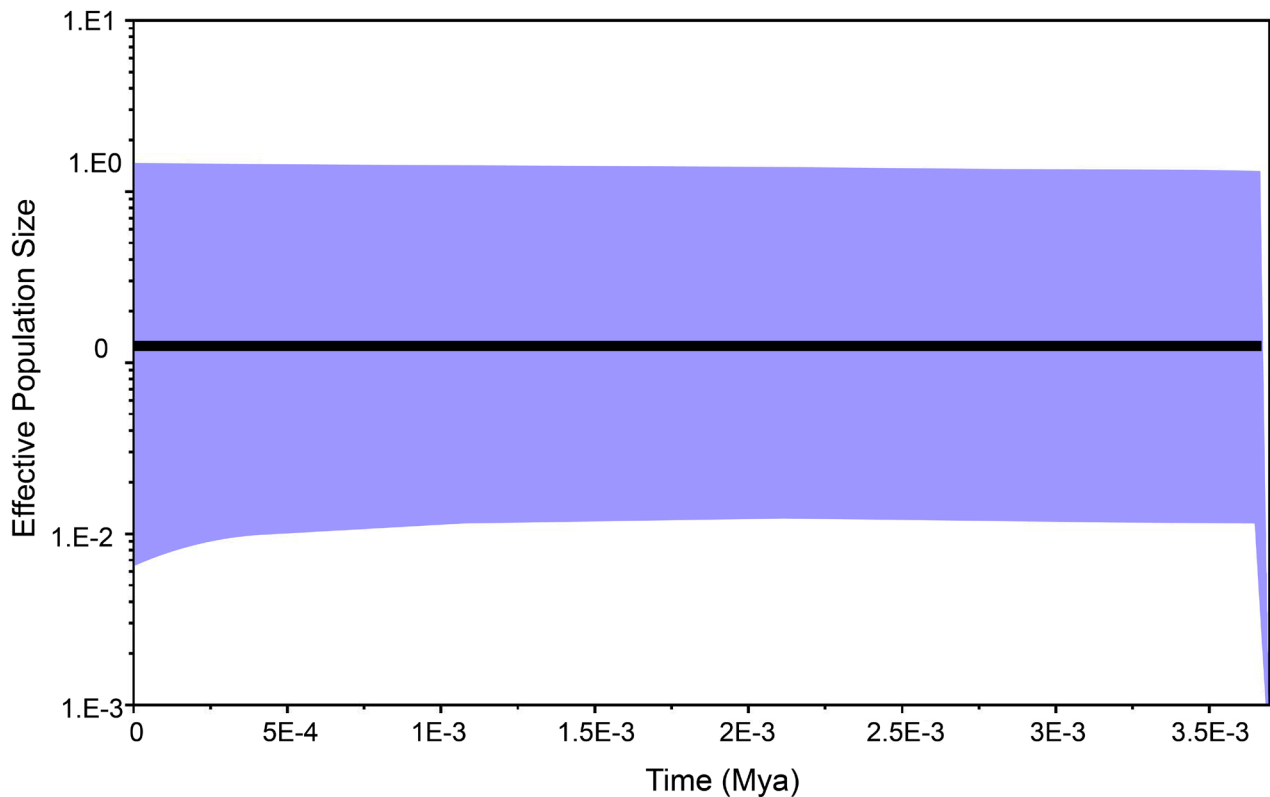


FIGURE 6 | Bayesian skyline plot showing the effective population size fluctuation of *Acyrtus simon* through time (Mya) (black line: median estimation; purple: confidence interval).

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 erythroptus* 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 erythroptus*, 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 two other 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*

sanguineus Müller & Troschel, 1843. The genera *Acyrtus* and *Arcos* were described in the same article (Schultz, 1944), based on *Acyrtus rubiginosus* and *Arcos erythroptus*, 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 erythroptus*, 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, 40.77–57.50 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.

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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).

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The authors declare no competing interests.

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