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Redescription of *Austrolebias accorsii* (Cyprinodontiformes: Rivulidae) and description of a new species of the genus from the upper Paraguay River basin

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Austrolebias accorsii is redescribed based on specimens from type-locality, nearby temporary pools, and other locations within the same hydrologic unit. Additionally, a novel *Austrolebias* species is described, exclusively found in two temporary waterbodies in "Abayoy", a unique vegetation type situated in the transitional zone between the Dry Chaco, Chiquitano Dry Forest, and Pantanal Flooded Savannas ecoregions in southeastern Bolivia, bordering Paraguay, where the last voluntarily isolated indigenous people outside the Amazon in America reside. Diagnostic characters for both species are presented. Within *Austrolebias* two species groups are recognized with different morphological characters, geographical distribution, and ecological traits of their habitats. Furthermore, we suggest the usage of median and interquartile range rather than mean and standard deviation to analyze morphometric and meristic data of rivulids, given the typically non-Gaussian distribution of this data.

Keywords: Chaco, Killifish, Seasonal fish, Taxonomy, Temporary water bodies.

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Austrolebias accorsii es redescrita basado en especímenes de la localidad tipo, charcos temporales cercanos y otras localidades en la misma unidad hidrológica. Además, se describe una nueva especie de *Austrolebias*, exclusiva de dos cuerpos de agua temporales en "Abayoy", un tipo de vegetación único situado en la zona de transición entre las ecorregiones del Chaco Seco, Bosque Seco Chiquitano y Sabanas Inundadas del Pantanal al sureste de Bolivia, en la frontera con Paraguay, donde residen los últimos indígenas en aislamiento voluntario fuera de la Amazonía en América. Se presentan caracteres diagnósticos para ambas especies. Dentro de *Austrolebias* se reconocen dos grupos de especies con diferentes caracteres morfológicos, distribución geográfica y características ecológicas del hábitat. Además, sugerimos el uso de mediana y rango intercuartil en lugar de media y desviación estándar para analizar datos morfométricos y merísticos de rivúlidos, dada la distribución generalmente no gaussiana de estos datos.

Palabras clave: Chaco, Cuerpos de agua temporales, Killifish, Peces estacionales, Taxonomía.

INTRODUCTION

The genus *Austrolebias* Costa, 1998, belonging to the family Rivulidae Myers, 1925, subfamily Cynolebiinae Hoedeman, 1961, was originally proposed to separate from *Cynolebias* Steindachner, 1876 a clade of two dozen morphologically and biogeographically distinguishable species (Costa, 1998). Subsequently, Costa (2006) synonymized the genus *Megalebias* Costa, 1998 (also previously proposed from a *Cynolebias* clade) into *Austrolebias*, encompassing 38 species organized into five species groups. In a subsequent revision, Costa (2008) redefined these clades, with some changes, as seven subgenera (*Acantholebias* Costa, 2008, *Acrolebias* Costa, 2008, *Argolebias* Costa, 2008, *Austrolebias*, *Cypholebias* Costa, 2008, *Gymnolebias* Costa, 2008, and *Megalebias*). The subgenus *Austrolebias* proposed by Costa (2008) initially comprised 14 species allocated into two species groups, the *A. adloffi* (Ahl, 1922) and *A. bellottii* (Steindachner, 1881) species groups. Loureiro *et al*. (2018), based on morphological and molecular characters confirmed with some discrepancies the subgenera proposed by Costa (2008) and recognized two additional clades left unnamed. Finally, Alonso *et al.* (2023) through a more comprehensive analysis of morphological and molecular characters, elevated the seven subgenera proposed by Costa (2008) to genus level and erected four new genera (*Amatolebias* Alonso, Terán, Serra, Calviño, Montes, Garcia, Barneche, Almirón, Ciotek, Giorgis & Casciotta, 2023; *Matilebias* Alonso, Terán, Serra, Calviño, Montes, Garcia, Barneche, Almirón, Ciotek, Giorgis & Casciotta, 2023; *Titanolebias* Alonso, Terán, Serra, Calviño, Montes, Garcia, Barneche, Almirón, Ciotek, Giorgis & Casciotta, 2023; and *Garcialebias* Alonso, Terán, Serra, Calviño, Montes, Garcia, Barneche, Almirón, Ciotek, Giorgis & Casciotta, 2023). Consequently, *Austrolebias sensu* Costa (2008) became a group of 11 different genera, one of which is *Austrolebias sensu stricto*, containing the species previously included in the *A. bellottii* species group and other species described later: *A. accorsii* Nielsen & Pillet, 2015, *A. bellottii*, *A. ephemerus* Volcan & Severo-Neto,

2019, *A. melanoorus* (Amato, 1986), *A. queguay* Serra & Loureiro, 2018, *A. univentripinnis* Costa & Cheffe, 2005, and *A. vandenbergi* (Huber, 1995) (Alonso *et al*., 2023).

The *Austrolebias* species can be distinguished from all other species of that group by the combination of the following characters: urogenital papilla attached to anal fin (except in *A. accorsii* and *A. vandenbergi*); anteromedial anal-fin rays elongated in females resulting in a triangular shaped anal fin; pectoral fins in males with a black margin and almost always an iridescent submargin; overlapping pelvic bones; absence of conspicuous and regular dark gray vertical bars on the flanks only anterior to the origin of the anal fin; absence of dark gray pigmentation in the cephalic neuromasts of the parietal series; and absence of black spots on the posterior flanks and vertically aligned in the caudal peduncle, in females (Alonso *et al*., 2023).

The main diagnostic characters of the *Austrolebias* species were established in the redescriptions of *A. bellottii*, *A. melanoorus*, *A. univentripinnis*, and *A. vandenbergi* by Costa (2006), and for subsequently described species, in the original descriptions of *A. accorsii* by Nielsen, Pillet (2015a), *A. queguay* by Serra, Loureiro (2018), and *A. ephemerus* by Volcan, Severo-Neto (2019). However, inconsistencies currently exist for some characters indicated in the description of *A. accorsii*, significantly hindering the unequivocal differentiation between this species and *A. vandenbergi*, or not corresponding with observations from photographs of the type material and topotypes of the species (Alonso *et al*., 2016; Drawert, 2023). Consequently, the current differentiation of these two species is often done, in practice, based on the geographical location of occurrence rather than considering morphological characters. To definitively address this issue, both Alonso *et al*. (2016) and Drawert (2023) recognized the need to redescribe *A. accorsii* based on a sufficiently large and representative set of specimens.

The geographical distribution of *Austrolebias* is confined to the Chacoan, Pampean, and Esteros del Ibera biogeographic provinces (*sensu* Morrone *et al*., 2022) in the Dry and Humid Chaco, Espinal, Humid Pampas, Paraná Flooded Savanna, and Uruguayan Savanna ecoregions (*sensu* Olson *et al*., 2001) of central and northern Argentina, southern Bolivia and Brazil, Paraguay, and Uruguay. They exclusively inhabit temporary aquatic ecosystems, preferably surrounded by open vegetation, within the hydrological systems of Laguna Merín (Jaguarão River basin); the upper Iténez/Guaporé River basin in the Amazon macrobasin; and the La Plata River macrobasin, encompassing the basins of the Paraguay River, lower La Plata River, lower Uruguay River (Tacuarembó and Queguay rivers), and lower Paraná River (Costa, 2006, 2008; Nielsen, Pillet, 2015a; Alonso *et al*., 2016; Loureiro *et al*., 2016; Serra, Loureiro, 2018; Volcan, Severo-Neto, 2019; Alonso *et al*., 2023; Drawert, 2023).

In the northern Chacoan biogeographic province, three *Austrolebias* species have been documented to date (Huber, 1995; Montaña *et al*., 2012; Nielsen, Pillet, 2015a; Alonso *et al*., 2016; Volcan, Severo-Neto, 2019). The northwestern sector, corresponding to the northern Dry Chaco ecoregion in Argentina, Bolivia, and Paraguay, is inhabited by *A. vandenbergi* in hydrological units (Bermejo, Monte Lindo, Pilcomayo, and Timané) belonging to the western part of the middle and upper Paraguay River basin (La Plata macrobasin) (Huber, 1995; Nielsen, Pillet, 2015a; Alonso *et al*., 2016). In Bolivia, in the northernmost section of the Dry Chaco ecoregion, where the transition to the Chiquitano Dry Forest ecoregion begins, and exclusively in the upper basin of the Iténez/Guaporé River (Amazon macrobasin), *A. accorsii* is found (Nielsen, Pillet, 2015a).

On the other side, *A. ephemerus* is recorded in the northeastern sector of the northern Chacoan biogeographic province, corresponding to the northern Humid Chaco ecoregion, solely in a few ephemeral pools in the drainage area of the Amonguijá River in the eastern part of the middle Paraguay River basin (La Plata macrobasin) in the state of Mato Grosso do Sul, Brazil (Volcan, Severo-Neto, 2019). In the section that separates the distribution areas of *A. ephemerus* and those of *A. accorsii* and *A. vandenbergi*, the presence of *Austrolebias* members has not been reported so far.

Herein, we redescribe *Austrolebias accorsii* based on specimens collected at the type-locality, nearby locations, and other sites within the same ecosystem and hydrological unit as the type material used in the original description by Nielsen, Pillet (2015a). Subsequently, we describe a new species belonging to the genus *Austrolebias*, found in temporary aquatic habitats of the San Miguel River hydrological unit in the upper Paraguay River basin (La Plata macrobasin). This new species is endemic to temporary water bodies in the "Abayoy", a unique vegetation type situated in the transitional sector between the Dry Chaco, the Chiquitano Dry Forest, and the Pantanal Flooded Savannas ecoregions in the eastern border region between Bolivia and Paraguay (Villarroel *et al*., 2022). Moreover, the area where the new species was discovered is the "Ñembi Guasu" (great hideout, in Guarani) of at least one group of Ayoréodes, the last indigenous people in voluntary isolation outside the Amazon in the Americas (Camacho, 2010; Cuéllar, Noss, 2014; Brackelaire, 2021; Flores *et al*., 2021).

MATERIAL AND METHODS

The specimens were captured using hand nets and gradually acclimated to clear water. Once adapted and stress-free, they were photographed alive. In the case of *A. accorsii*, specimens were transported alive to Santa Cruz and photographed under controlled light conditions (LED 50 W, 4000 lm, 6500° K, CRI > 80). Specimens of the new species were acclimated to clear water and photographed in the field due to the difficult access and remoteness of the collection locality. Subsequently, specimens were euthanized with a 25 ml/l solution of ethyl-alcoholic clove extract (*Syzygium aromaticum* Merr & Perry, 1939 flower buds), fixed in 4% formaldehyde for one week, and transferred to 70% ethanol for preservation; or preserved directly in 96% ethanol without fixation. Morphometric measurements were taken point to point with a digital caliper (precision ± 0.02 mm) and rounded to the nearest 0.1 mm following the landmarks proposed by Costa (1995) with the adjustments indicated by Serra, Loureiro (2018). Standard length (SL) is indicated in millimeters, and the rest of the measurements are presented as percentages relative to SL, except those relating to the head, which are expressed as percentages relative to head length (HL). Scale and fin ray counts followed Costa (1995), including all visible elements for the fins. For the terminology of frontal head squamation, the proposed by Hoedeman (1958) was followed. Counting of cephalic neuromasts and nomenclature follows Costa (2001). Two males and one female of both species were cleared and stained (c&s) for the observation of osteological characteristics using a method by C. Ergueta (work in progress) for the diaphanization of small vertebrates, consisting of clearing with household degreasers and staining with annatto (*Bixa* sp., also known as achiote or urucú). To clear the samples, previously fixed and

conserved in 70% ethanol, an initial rinse with water was performed for 24 to 48 h, followed by evisceration and removal of scales. Subsequently, the samples were immersed in household degreaser ("Todo Brillo"® in this case), containing surfactants, organic solvents and sodium hydroxide, mixed with annatto as dye (one teaspoon per 250 ml). After 72 to 96 h the cleared and stained samples were transferred to pure glycerin for preservation. Vertebral counts, considering the compound caudal centrum as a single element, and gill raker counts were conducted exclusively on cleared specimens. The description of live coloration patterns was based on photographs taken from one or both sides of the body as indicated above.

References to ecoregions are made using the classification and names assigned by Olson *et al*. (2001), for biogeographic provinces Morrone *et al*. (2022) is used, and for lower biogeographic units and within the Bolivian territory, Navarro, Ferreira (2009) and Navarro, Maldonado (2002). Pfafstetter codes determined by Lehner, Grill (2013) are used for the identification of hydrological units (HU), and when available, names of water bodies and basins assigned by SUNIT (2007) are followed. Climatic data (ranges of minimum and maximum annual temperatures, and monthly and annual accumulated precipitation) for the occurrence locations were obtained from NASA (2023) based on a 41-year range (1981–2022). The nomenclature of institutions and collections is adhered to by either using their self-designated names in their original language or employing the acronyms as specified by Fricke, Eschmeyer (2023).

The material examined for the redescription of *A. accorsii* is presented including the full collection data. Since this is a redescription and there is no new type material designed for the species, the description is based on comparative material. The inclusion of full collection data allows inferring, at least approximately, the distance between the type-locality and the location of the collected material. The collected material is deposited in the ichthyological collection of the Museo de Historia Natural Noel Kempff Mercado (MHNNKM) in Santa Cruz de la Sierra, Bolivia. Morphological, morphometric, meristic, osteological characters, and coloration patterns of other *Austrolebias* were obtained from original descriptions or redescriptions of the species (Amato, 1986; Huber, 1995; Braga, 1999; Costa, 2006; Nielsen, Pillet, 2015a; Serra, Loureiro, 2018; Volcan, Severo-Neto, 2019; Serra *et al*., 2022). Additional comparative material examined is detailed in the corresponding section at the end of the Discussion.

RESULTS

Austrolebias accorsii Nielsen & Pillet, 2015

(Figs. 1–2; Tab. 1)

Austrolebias accorsii Nielsen, Pillet, 2015a:174, figs. 1–2 (photos, original description). —Nielsen, Pillet, 2015b:186 (list of syntopical species). —Alonso *et al*., 2016:5 (comparison). —Alonso *et al*., 2018:2 (mention). —Serra, Loureiro, 2018:547 (mention). —Volcan, Severo-Neto, 2019:542 (mention). —Zamorano, 2019:38 (list of new species for Bolivia). —Alonso *et al*., 2023:2 (mention).

Austrolebias vandenbergi Huber, 1995. —Montaña *et al*., 2012:589, fig. 1 (photo, misidentified specimens from Santa Cruz, Bolivia).

Austrolebias vandengerbi. —Montaña *et al*., 2012:590 (misspelled).

Diagnosis. Males of *Austrolebias accorsii* differ from all congeners, except *A. vandenbergi*, by absence of whitish to light blue dots or bars on flanks (*vs*. presence of vertical rows of dots in *Austrolebias* n. sp. (described below), *A. bellottii*, and *A. univentripinnis*; vertical bars in *A. ephemerus*, *A. melanoorus*, and *A. queguay*) and by urogenital papilla not attached to anal fin; and, except *Austrolebias* n. sp., *A. ephemerus*, and *A. vandenbergi*, by presence of tree or more transverse rows of scales on basal anterior and median portion of anal fin. It can be distinguished from *A. vandenbergi* by the absence of white or whitish dots on unpaired fins (*vs*. presence), presence of well-defined black marginal line on anal fin (*vs*. if dark margin present, broader and attenuated), and dark blotch on dorsum between end of head and dorsal-fin origin (*vs.* absent or inconspicuous). Males of *A. accorsii*, as well, differ from those of *A. queguay* by having more anal-fin rays (28–36 *vs*. 24–27); and from *A. melanoorus* by more longitudinal series of lateral scales (30–34 *vs*. 26–29) and more posterior dorsal-fin origin (vertical between 4th and 6th anal-fin ray *vs*. anterior to anal-fin origin). Females of *A. accorsii* can be distinguished from all other congeners, except *Austrolebias* n. sp. and *A. ephemerus*, by a more posterior dorsal-fin origin (vertical between 5th and 10th anal-fin ray *vs*. anterior to 5th anal-fin ray); from *A.melanoorus*, *A. queguay*, *A. univentripinnis*, and *A. vandenbergi* by posterior tip of pectoral fins reaching vertical between 3rd and 7th anal-fin ray (*vs*. anterior to 2nd anal-fin ray); from *A. melanoorus* and *A. univentripinnis* by a longer prepelvic length (41.3–48.5% SL *vs*. 52.6–59.2%, 52.5–57.7%, respectively); from *A. queguay* by having more anal-fin rays (25–35 *vs*. 21–24).

Description. Morphometric and meristic data presented in Tab. 1. Males larger than females; largest examined male 55.2 mm SL, largest female 42.6 mm SL. Body elongated, moderately deep and laterally compressed. Highest body depth approximately on vertical at anal-fin origin. On lateral view, dorsal profile of head straight to slightly concave from snout to vertical between eye and superior limit of operculum, from this point to posterior end of dorsal-fin base convex, and straight to slightly concave on caudal peduncle; ventral profile slightly convex from lower jaw to pelvic-fin base, from there to end of anal-fin base straight to slightly convex, and straight to slightly concave on caudal peduncle. Snout blunt and jaws short.

Tip of dorsal and anal fins rounded in both sexes; in females, anteromedial anal-fin rays lengthened resulting in triangular shaped anal fin; dorsal-fin origin on vertical between 4th and 6th anal-fin ray in males, and 5th to 10th in females; dorsal-fin origin between neural spines of 9th to 10th vertebrae in males and 10th to 11th in females; anal-fin origin between pleural ribs of 6th to 8th vertebrae in males and 5th to 6th in females. Caudal fin rounded. Pectoral fin elliptical; posterior margin on vertical between 4th and 7th anal-fin ray in males, between 3rd and 7th in females. Pelvic fin membrane medially coalesced 50–75% of length, posterior tip reaching between 1st and 4th anal-fin ray in males, and between 2nd and 3rd in females. Urogenital papilla in males attached to anal fin only basally and maximum 10% of length, prominent in females. Dorsal-fin rays 22–32 in males and 20–27 in females; anal-fin rays 28–36 in males and 25–35 in females; caudal-fin rays 20–26 in males and 24–28 in females; pectoral-fin rays 12–14 in males and 11–13 in females; pelvic-fin rays 4–5 (one male with 3 and two with 7, one female with 6) in both sexes.

TABLE 1 | Morphometric and meristic data of *Austrolebias accorsii*. 1: MNKP 16552 (topotypes, n = 5), MNKP 16556 (n = 6), MNKP 16584 (n = 2); 2: MNKP 16552 (topotypes, n = 5), MNKP 16556 (n = 5), MNKP 16584 (n = 1).

	Austrolebias accorsii					
	Males[1] (n = 13)			Females[2] (n = 11)		
	Min–Max	Median (IQR)	Mean (SD)	Min–Max	Median (IQR)	Mean (SD)
Standard length (mm)	32.42–55.17	40.58 (7.26)	41.52 (6.26)	29.22–42.61	33.72 (4.3)	33.75 (4.26)
Percents of standard length						
Body depth	35.01–43.92	41.37 (4.22)	40.28 (3.11)	35.53–46.96	41.17 (3.24)	41.36 (3.18)
Caudal peduncle depth	13.5–16.17	14.96 (1.41)	15.01 (0.83)	12.76–16.53	14.33 (1.99)	14.7 (1.26)
Pre-dorsal length	48.77–55.64	51.47 (3.58)	51.93 (2.34)	48.96–60.98	58.72 (1.69)	57.85 (3.52)
Pre-pelvic length	38.59–46.1	41.64 (2.2)	41.42 (2.03)	41.3–48.54	45.38 (2.56)	45.86 (2.33)
Dorsal-fin base length	35.1–43.37	38.94 (2.71)	38.58 (2.44)	23.7–37.6	27.32 (2.01)	27.85 (3.67)
Anal-fin base length	35.61–52.9	44.73 (6.49)	45.75 (4.52)	27.92–40.18	31.79 (3.2)	32.16 (3.24)
Caudal-fin length	13.5–23.5	20.21 (4.34)	19.9 (2.98)	22.32–29.06	25.73 (1.96)	25.42 (1.75)
Pectoral-fin length	18.75–25.56	21.87 (2.03)	21.99 (1.99)	18.36–27.34	22.84 (2.9)	22.95 (2.84)
Pelvic-fin length	8.58–13.02	9.65 (0.89)	9.97 (1.42)	10.21–16.11	14.54 (3.5)	13.55 (2.06)
Head length	26.37–31.3	28.44 (1.07)	28.55 (1.44)	27.92–34.79	29.72 (3)	30.88 (2.33)
Percents of head length						
Head depth	102.14–124.81	109.29 (12.38)	110.53 (8.06)	79.05–109.82	104.31 (7.89)	102.04 (9.1)
Head width	58.2–75.53	66.51 (6.83)	67.23 (5.02)	57.84–73.74	65.47 (4.7)	64.95 (4.57)
Snout length	18.39–31.11	24.54 (3.49)	24.29 (3.68)	16.93–24.73	23.15 (2.72)	22.1 (2.76)
Eye diameter	21.18–30.66	26.35 (2.58)	26.24 (2.42)	21.27–35.22	27.46 (2.87)	28.02 (4)
Meristic counts						
Longitudinal series scales	30–34	33 (1)	32.62 (1.19)	30–34	32 (1.5)	32.36 (1.43)
Transverse series scales	14–18	16 (1)	16.23 (1.17)	14–18	16 (1.5)	16.09 (1.14)
Circumpeduncular rows scales	16–20	18 (0)	18 (1.15)	16–21	19 (1.5)	18.82 (1.33)
Pectoral fin rays	12–14	12 (0)	12.31 (0.63)	11–13	12 (0)	12 (0.45)
Pelvic fin-rays	4–6	5 (1)	4.69 (0.75)	4–6	5 (0)	5 (0.45)
Dorsal fin-rays	22–32	26 (3)	25.92 (2.78)	20–27	22 (4)	22.45 (2.66)
Anal fin-rays	28–36	34 (2)	33.23 (2.28)	25–35	30 (6)	30.36 (3.56)
Caudal fin-rays	20–26	24 (2)	24.31 (1.75)	24–28	26 (2)	25.64 (1.5)

Scales cycloid, large. Trunk and head completely scaled, except ventral surface of head. Frontal squamation H-patterned or sometimes G-patterned (two males), E-scales usually overlapping medially; scales transversally arranged on trunk. No scales surpassing base of dorsal, pectoral, and pelvic fins in both sexes; in males 3 to 6, and in females 1 to 3, rows of scales over-passing anal-fin base on anterior and median portion; 3 rows of scales on caudal-fin base in both sexes. Longitudinal series of scales 30–34, transversal series of scales 14–18, and circumpeduncular rows of scales 16–20 (one female with 21), in both sexes. Males with 1–3 contact organs per scale on anterior central and ventral section of flanks and opercular region; rows of contact organs on 3–4 uppermost pectoral-fin rays and on anterior distal anal-fin rays; no contact organs on other fins and not present on females.

Cephalic neuromasts: supraorbital 14–19 (usually 16–18), parietal 1–4 (usually 2), anterior rostral 1–2 (usually 2, sometimes 3 adding both sides of head), posterior rostral 1–2 (usually 1), infraorbital 2 + 24–26, preorbital 2–3 (usually 2), otic 1–4 (usually 3), post-otic 4–5, lateral supratemporal 1–2 (usually 1), median opercular 1, ventral opercular 1–4 (usually 2–3), preopercular 21–25, mandibular 13–16 (usually 14), lateral mandibular 4–8 (usually 7), post-temporal 1–2 (usually 1). Lateral line complete, with one neuromast per scale.

Total number of vertebrae 30–32. Hypurals ankylosed in a single plate without visible fissure. Six branchiostegal rays, arranged in 3 pairs. Gill-rakers on 1st branchial arch 6–8 + 14.

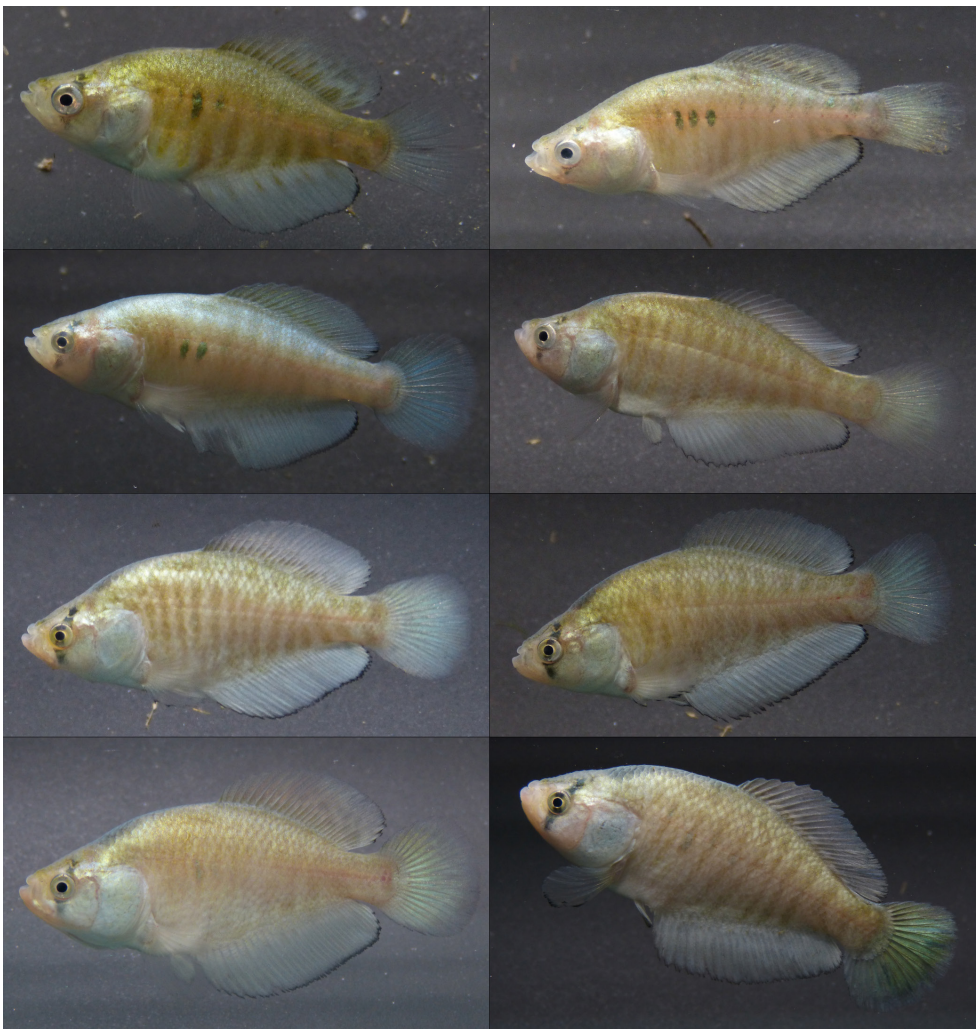

FIGURE 1 | *Austrolebias accorsii*. Males (1 day after collection). Left: MNKP 16556, 32.4–42.3 mm SL, Bolivia, Santa Cruz, Cordillera, Charagua Iyambae Guarani Autonomous Territory. Right: MNKP 16552, topotypes, 39.3–48.6 mm SL, Bolivia, Santa Cruz, Cordillera, Charagua Iyambae Guarani Autonomous Territory.

Coloration in life. Males (Fig. 1). Head greenish gray, opercular region bluish gray. Black bar crosses vertically the eye; subrectangular at suborbital portion with tip directed slightly posteroventrally, and subtriangular at supraocular portion slightly surpassing dorsally the line of parietal neuromasts. Iris whitish gray to pale yellow with black bar through center of eye. Trunk and caudal peduncle ground color greenish gray to greenish pale brown, darker on dorsal region and paler on anteroventral region. Neck notoriously dark. Subadults usually with golden-metallic shine on anterior dorsal region. Flanks and caudal peduncle sides with 8–17 dark vertical bars of same color as most dorsal portion of flanks, usually curved on posterior region of flanks and caudal peduncle; conspicuous on juveniles and fading with age, almost disappearing in senescent specimens. Sometimes one or two dark greenish metallic blue, vertically elongated, spots on anteromedial region of flanks, approximately on or some anterior to center of imaginary axis between origins of dorsal and anal fins, fading with age and absent in older males. Pectoral fins hyaline, ventrally, and sometimes also distally, with black margin. Pelvic fins whitish to gray, usually with black tips. Dorsal fin greenish gray on basal half and more bluish gray on distal, sometimes with rosy hue, and usually with black posterior distal margin; in subadults distal half hyaline to pale gray. Anal fin gray to bluish gray with conspicuous black distal margin. Caudal fin light blue to pale turquoise, sometimes hyaline distally, especially in subadults.

Females (Fig. 2). Head greenish yellow to yellowish brown, opercular region pale bluish gray to greenish gray. Sometimes inconspicuous dark vertical bar through eye. Iris whitish gray to pale yellow with dark bar through center of eye. Trunk and caudal peduncle ground color greenish pale yellow to yellowish brown, anteroventral region pale yellow to whitish. Flanks and caudal peduncle sides with dark, sometimes bluish metallic shining, vertically elongated spots, in some cases at medial flanks coalesced forming vertical bars between dorsal and anal fin bases. On anteromedial flanks, some anterior to the center of the linear axis between anal- and dorsal-fin bases, 1–4 black, sometimes greenish to bluish iridescent, vertical elongated blotches. Fins hyaline; dorsal fin sometimes pale yellowish at basal half and/or with some black dots; anal fin sometimes with pale light blue shine, and/or pale yellowish at anterior and median basal section and with some black dots on posterior basal section.

Coloration in alcohol. Overall color pattern similar to that of live specimens, but less intense and faded, without iridescence and shines, or color hue. Body background color yellowish gray to pale yellowish brown in both sexes.

Geographical distribution. *Austrolebias accorsii* is the only species of the genus found in the Amazon basin, and with distribution outside the La Plata basin. The distribution is restricted to the upper Iténez/Guaporé River drainage (hydrologic unit HU 62268), southern of the Chiquitos montain range, in and around the "Bañados del Izozog" wetlands in the watersheds of the Tunas (HU 6226858) and Quimome (HU 622689 and 622688) rivers. Therefore, the species is endemic to this area in the Cordillera Province of Santa Cruz Department in Bolivia (Fig. 3).

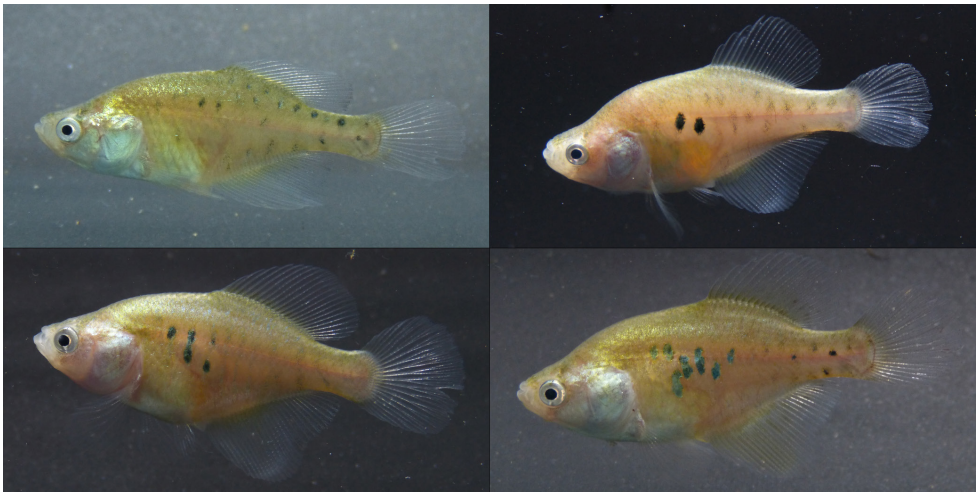

FIGURE 2 | *Austrolebias accorsii*. Females (1 day after collection). MNKP 16552, topotypes, 33.72–42.61 mm SL, Bolivia, Santa Cruz, Cordillera, Charagua Iyambae Guarani Autonomous Territory.

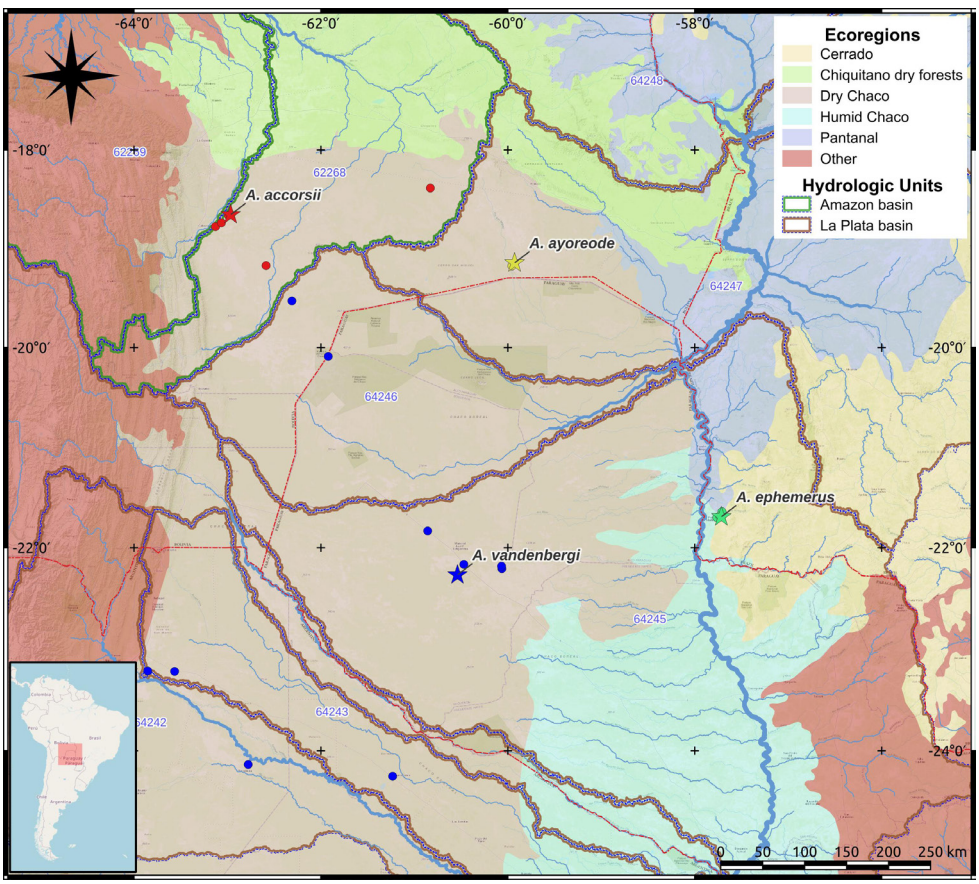

FIGURE 3 | Occurrence localities of *Austrolebias* species in the northern Chacoan province. Stars represent type-localities, colors differentiate the species (*A. accorsii* red, *Austrolebias* n. sp. yellow, *A. ephemerus* green, *A. vandenbergi* blue). Blue numbers indicate the Hydrologic Unit (Pfafstetter, level 5). Sources: Ecoregions from Olson *et al.* (2001); Hydrologic Units from Lehner, Grill (2013); Basemap ESRI World Topo (2022).

Ecological notes. The species is exclusively found in temporary pools with muddy waters in the dry Chaco forests of the northern section of the Chacoan biogeographic province, in the Izozog district (Fig. 4). The depth of these water bodies does not exceed 50 cm, and the substratum typically consists of a thin layer of plant litter and debris (1 to 2 cm) on a clay bottom. The pools lack submerged aquatic vegetation but feature floating and emergent vegetation, mainly from the Poaceae and Polygoniaceae families, typically limited to the riparian zone. The only sympatric fish species found in these aquatic ecosystems are other rivulids, such as *Neofundulus* aff. *paraguayensis* (Eigenmann & Kennedy, 1903), *Titanolebias monstrosus* (Huber, 1995), *Trigonectes aplocheiloides* (Huber, 1995), and in the northeasternmost locations, also *Spectrolebias brousseaui* Nielsen, 2013. The annual minimum and maximum temperatures range from 1.9–9.9°C to 34.8–43.8°C (averages of 5.7–6.2°C and 39.2–40.6°C for different locations), with minimums concentrated in the months of May to August and maximums in September to November. Annual accumulated precipitation in different locations varies from 564–633 mm to 1366–1566 mm (averages of 886–1018 mm), predominantly concentrated in the months of December to March.

Conservation status. The species has an estimated extent of occurrence (EOO) of less than 20,000 km^2. The estimation of the area of occupancy (AOO) is complex, considering that this species inhabits small ephemeral and temporary aquatic ecosystems (often less than 100 m^2) in the dry forest of the northern Chacoan biogeographic province, which are generally scattered, isolated, and challenging to quantify using telemetric methods.

FIGURE 4 | Biotope at type-locality of *Austrolebias accorsii*. Bolivia, Santa Cruz, Cordillera, Charagua Iyambae Guarani Autonomous Territory, 18°39'26.2"S 62°58'00.2"W, 30 April 2021.

This indicates a severely fragmented distribution, further exacerbated by the fact that there are only three known subpopulations of the species in two locations. Although approximately 50% of the EOO is within the Kaa-Iya del Gran Chaco National Park and Integrated Management Natural Area, the other half faces significant pressure on natural ecosystems. In the last 25 years, and more intensely in the last 10 years, these ecosystems have been replaced by large-scale agro-industrial crops over extensive areas. It is inferred that this trend of advancing agricultural frontiers, especially in the western and northern parts of the EOO, will continue or even accelerate in the coming years due to recent government policies promoting the expansion of agricultural activities in the region. The announced introduction of genetically modified drought-resistant crops in the near future poses a significant threat, as adverse climatic conditions for conventional crops have so far been the primary obstacle to the expansion of industrialized agriculture in the Dry Chaco ecoregion of Bolivia. On the other hand, the direct and indirect effects of climate change, such as changes in hydrological regimes or an increased incidence of wildfires, also represent potential threats to the conservation status of this species. Following the guidelines for categories and criteria of the International Union for Conservation of Nature (IUCN), the species may be considered Vulnerable (VU) as it meets criteria A3c, A4c, B1ab, and D2 (IUCN Standards and Petitions Committee, 2022).

Material examined. ZUEC 10792, holotype (photo only), male, 37.7 mm SL, Bolivia, Santa Cruz, [Charagua Iyambae Guarani Autonomous Territory], temporary pool, [hydrologic unit 6226858 (Pfafstetter), Parapetí watershed, Iténez/Guaporé drainage, Amazon basin (corrected)], 18°39'26.2"S 62°58'00.2"W, 10 Apr 2014, V. Chaverou, B. Accorsi, M. Beuchey, S. Blois, E. Vandekerkhove & D. Pillet. ZUEC 10793, paratype (photo only), 1 female, 39.2 mm SL, collected with holotype. **Non-types:** MNKP 16550, 3 (juveniles), Bolivia, Santa Cruz, Charagua Iyambae Guarani Autonomous Territory, temporary pool near Izozog on road from Abapó to Pailón, hydrologic unit 6226858 (Pfafstetter), Parapetí watershed, Iténez/Guaporé drainage, Amazon basin, 18°46'42"S 63°07'44"W, 30 Apr 2021, H. A. Drawert & J. C. Catari. MNKP 16551, 3 (2 males, 1 female), Bolivia, Santa Cruz, Charagua Iyambae Guarani Autonomous Territory, temporary pool near Izozog on road from Abapó to Pailón, hydrologic unit 6226858 (Pfafstetter), Parapetí watershed, Iténez/Guaporé drainage, Amazon basin, 18°44'24"S 63°03'57"W, 30 Apr 2021, H. A. Drawert & J. C. Catari. MNKP 16552, topotypes, 23 (7 males, 16 females), 39.3–48. 6 mm SL (males) 33.7–42.6 mm SL (females), Bolivia, Santa Cruz, Charagua Iyambae Guarani Autonomous Territory, temporary pool near Izozog on road from Abapó to Pailón, hydrologic unit 6226858 (Pfafstetter), Parapetí watershed, Iténez/Guaporé drainage, Amazon basin, 18°39'27"S 62°58'00"W, 30 Apr 2021, H. A. Drawert & J. C. Catari. MNKP 16553, topotypes, 4 (1 male, 3 females), collected with MNKP 16552. MNKP 16556, 16 [7 (2 c&s) males, 9 (1 c&s) females], 32.4–42.3 mm SL (males) 29.2–31.6 mm SL (females), Bolivia, Santa Cruz, Charagua Iyambae Guarani Autonomous Territory, temporary pool near Izozog on road from Abapó to Pailón, hydrologic unit 6226858 (Pfafstetter), Parapetí watershed, Iténez/Guaporé drainage, Amazon basin, 18°39'49"S 62°59'31"W, 30 Apr 2021, H. A. Drawert & J. C. Catari. MNKP 16583, 6, Bolivia, Santa Cruz, Charagua Iyambae Guarani Autonomous Territory, Ñembi Guasu Conservation and Ecological Importance Area, temporary pool near Tucabaca Camp on road to San

José de Chiquitos, hydrologic unit 6226882 (Pfafstetter), Parapetí watershed, Iténez/Guaporé drainage, Amazon basin, 18°23'15"S 60°49'48"W, 4 Jun 2021, C. Ergueta & H. A. Drawert. MNKP 16584, 3 (2 males, 1 female), 43.8–55.2 mm SL (males), 39.8 mm SL (female), Bolivia, Santa Cruz, Charagua Iyambae Guarani Autonomous Territory, Ñembi Guasu Conservation and Ecological Importance Area, temporary pool near Tucabaca Camp on road to San José de Chiquitos, hydrologic unit 6226882 (Pfafstetter), Parapetí watershed, Iténez/Guaporé drainage, Amazon basin, 18°23'38"S 60°49'38"W, 4 Jun 2021, C. Ergueta & H. A. Drawert.

Austrolebias ayoreode, new species

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(Figs. 5–7; Tab. 2)

Holotype. MNKP 16612, male, 50.7 mm SL, Bolivia, Santa Cruz, Charagua Iyambae Guarani Autonomous Territory, Ñembi Guasu Conservation and Ecological Importance Area, temporary pool within low dry forest (Abayoy) on side of dirt road between San Jorge and Ponderosa ranches *ca*. 10 km west of San Jorge, hydrologic unit 6424726 (Pfafstetter), San Miguel watershed, upper Paraguay River drainage, La Plata basin, 19°08'46"S 59°55'44"W, 10 Jun 2021, C. Ergueta & H. A. Drawert.

Paratypes. MNKP 16587, 12 males (21.2–34.7 mm SL), 11 females (19.7–38.1 mm SL), collected with holotype. MNKP 16590, 2 males (34.7–34.8 mm SL), 1 female (26.1 mm SL), c&s, collected with holotype.

Non-types. MNKP 16589, female, Bolivia, Santa Cruz, Charagua Iyambae Guarani Autonomous Territory, Ñembi Guasu Conservation and Ecological Importance Area, temporary pool within low dry forest (Abayoy) *ca*. 1 km southwest of Ponderosa ranch, hydrologic unit 6424726 (Pfafstetter), San Miguel watershed, upper Paraguay River drainage, La Plata basin, 19°10'35"S 59°57'24"W, 10 Jun 2021, C. Ergueta.

Diagnosis. *Austrolebias ayoreode* males differs from all congeners, except *A. accorsii*, *A. ephemerus*, and *A. vandenbergi*, by presence of 1–3 transverse rows of scales on basal anterior and median portion of anal fin. Males of *A. ayoreode* can be distinguished from *A. accorsii*, *A. ephemerus*, *A. melanoorus*, *A. queguay*, and *A. vandenbergi* by presence of whitish to pale yellowish dots on flanks usually vertically aligned in unarranged rows (*vs*. absent in *A. accorsii;* mostly coalesced in vertical bars in *A. ephemerus*, *A. melanoorus*, and *A. queguay*; absent or, when rarely present, sparse and scattered in *A. vandenbergi*); from *A. accorsii* and *A. vandenbergi* by urogenital papilla mostly attached to anal fin (70–80% adhered *vs*. only on base and maximum 10% adhered), medial pelvic fin membranes 75–100% coalesced (*vs*. less than 75% coalesced), and less transversal series of scales (11–13 *vs*. 14–18, 13–18 [14–17 on comparative material examined, Tab. **S1**], respectively); from *A. ephemerus* by shorter pelvic fin (5.0–8.1% SL *vs*. 8.6–11.1%), wider head width (65.0–83.5% SL *vs*. 55.3–61.1%), and more anterior rostral neuromasts (2–3 *vs*. 1); from *A. melanoorus* and *A. univentripinnis* by lower pre-pelvic length (38.4–46.7%

SL *vs*. 48.8–52.8%, 47.0–49.4%, respectively); from *A. melanoorus* by more longitudinal series of scales (30–33 *vs*. 26–29), more posterior dorsal-fin origin (vertical between of 4th and 5th anal-fin ray *vs*. anterior of 1st anal-fin ray); from *A. univentripinnis* by posterior tip of pelvic fins reaching between 1st and 2nd anal-fin ray (*vs*. between 3rd and 4th anal-fin ray); from *A. vandenbergi* by shorter body depth (32.6–38.6% SL *vs*. 39.6–46.7%). Females of the new species differ from *A. bellottii*, *A. melanoorus*, *A. univentripinnis*, and *A. vandenbergi* by more posterior dorsal-fin origin (vertical between 4th and 5th anal-fin ray *vs*. anterior of 4th anal-fin ray); from *A. melanoorus* and *A. univentripinnis* by shorter pre-dorsal length (49.0–58.1% SL *vs*. 60.8–64.4%, 58.7–63.5%, respectively); from *A. melanoorus*, *A. queguay*, and *A. univentripinnis* by posterior tip of pectoral fin reaching between 1st and 4th anal-fin ray (*vs*. not reaching over anal-fin origin); from *A. melanoorus* and *A. univentripinnis* by shorter pre-pelvic length (42.6–48.3% SL *vs*. 52.6–59.2%, 52.5–57.7%, respectively); from *A. bellottii* by shorter length of anal-fin base (23.7–29.9% SL *vs*. 32.6–38.1%) and shorter pelvic-fin length (6.6–12.2% SL *vs*. 12.3–16.8%); from *A. ephemerus* by greater head width (66.8–82.3% SL *vs*. 58.4–62.8%) and by posterior tip of pectoral fin reaching between 1st and 4th anal-fin ray (*vs*. reaching between 5th and 7th anal-fin ray); from *A. queguay* by higher anal-fin rays count (28–32 *vs*. 24–27).

Description. Morphometric and meristic data presented in Tab. 2. Males larger than females; largest examined male 50.7 mm SL, largest female 38.1 mm SL. Body elongated, moderately deep, and laterally compressed. Highest body depth approximately on vertical between origins of dorsal and anal fins in males, and on vertical through pelvic-fin base in females. On lateral view, dorsal profile of head in males slightly convex to almost straight, and in females straight to slightly concave, from snout to vertical between eye and superior limit of operculum, from this point to posterior end of dorsal-fin base convex with increased curvature, and on caudal peduncle straight; ventral profile convex from lower jaw to pelvic-fin base, from there to end of anal-fin base in males slightly convex to almost straight and in females straight to slightly concave, and straight on caudal peduncle. Snout blunt, mouth superior and jaws short.

Tip of dorsal and anal fins rounded in both sexes; in females, anteromedial anal-fin rays longer than the remaining rays, resulting in triangular shaped anal fin; dorsal-fin origin on vertical between 4th and 5th anal-fin ray; dorsal-fin origin between neural spines of 8th to 9th vertebrae in males and 11th to 12th in females; anal-fin origin between pleural ribs of 5th to 6th vertebrae in males and 7th to 8th in females. Caudal fin rounded. Pectoral fin elliptical; posterior margin reaching vertical between 4th and 6th anal-fin ray in males, and between 1st and 4th in females. Pelvic fins membranes medially coalesced in 75–100% of its length, posterior tip reaching between 1st and 2nd anal-fin ray when folded. Urogenital papilla in males attached to anal fin in 70–80% of its basal portion. Prominent in females. Dorsal-fin rays 23–29 in males and 18–22 in females; anal-fin rays 28–32 in males and 24–28 in females; caudal-fin rays 23–26; pectoral-fin rays 12–13; pelvic-fin rays 4–5.

Scales cycloid, large. Trunk and head completely scaled, except ventral surface of head. Frontal squamation H-patterned, no scales anterior to H-scale, E-scales usually overlapping medially; scales transversally arranged on trunk. In both sexes, no scales surpassing base of dorsal, pectoral, and pelvic fins; up to 3 rows of scales surpassing anal-

TABLE 2 | Morphometric and meristic data of *Austrolebias ayoreode*. 1: MNKP 16612; 2: MNKP 16587 (n = 12), MNKP 16590 (n = 2); 3: MNKP 16587 (n = 9), MNKP 16590 (n = 1); 4: Holotype and paratypes; 5: Females n = 9.

	Holotype	Paratypes		Males[4] (n = 15)		Females[3] (n = 10)	
	Male[1]	Males[2] (n = 14)	Females[3] (n = 10)				
		Min–Max	Min–Max	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)
Standard length (mm)	50.74	21.2–34.78	19.69–38.08	26.64 (7.13)	28.81 (7.46)	22.16 (2.09)	23.83 (5.32)
Percents of standard length							
Body depth	35.95	32.57–38.63	33.38–38	36.31 (1.88)	36.39 (1.53)	35.37 (1)	35.41 (1.31)
Caudal peduncle depth	14.37	13.29–16.51	10.49–16.36	14.45 (0.82)	14.65 (0.96)	13.82 (1.28)	13.55 (1.51)
Pre-dorsal length	49.94	45.31–54.33	49.02–58.11	47.47 (3.5)	48.47 (2.63)	54.16 (4)	54.26 (2.85)
Pre-pelvic length	38.39	39.02–46.71	42.56–48.3	42.18 (2.44)	41.93 (2.2)	44.82 (2.91)	45.08 (1.91)
Dorsal-fin base length	41.17	33.68–44.28	24.02–31	40.88 (2.35)	39.98 (3.05)	28.78 (2.59)	28.32 (2.21)
Anal-fin base length	45.92	39.04–45.17	23.72–29.92	42.95 (2.14)	42.46 (2.05)	27.29 (2.03)	27.48 (1.76)
Caudal-fin length[5]	18.58	18.34–26.14	18.72–26.22	22.12 (3.41)	21.6 (2.38)	21.78 (3.69)	22.29 (2.5)
Pectoral-fin length	22.09	18.88–27.5	19.81–25.06	22.59 (2.61)	22.58 (2.25)	22.82 (3.3)	22.36 (1.99)
Pelvic-fin length	8.10	5.05–7.93	6.56–12.18	6.27 (1.71)	6.55 (1.06)	9.01 (1.23)	9.04 (1.57)
Head length	28.42	24.96–32.55	26.48–31.79	28.12 (0.98)	28.25 (2)	29.05 (2.95)	29.64 (1.94)
Percents of head length							
Head depth	108.46	100.12–117.68	96.68–107.36	108.46 (6.87)	108.28 (5.2)	103.76 (4.81)	103.39 (3.33)
Head width	65.05	70.08–83.55	66.76–82.34	77.61 (9.36)	76.25 (5.55)	72.49 (6.97)	72.26 (4.93)
Snout length	23.58	14.78–25.18	15.77–23.27	21.81 (4.47)	20.72 (3.29)	20.43 (1.5)	19.94 (2.43)
Eye diameter	24.34	25–35.81	27.18–34.14	27.85 (5.49)	29.5 (3.63)	31.88 (3.09)	31.22 (2.24)
Meristic counts							
Longitudinal series scales[5]	30	30–33	30–31	31 (1.5)	31 (0.93)	31 (1)	30.56 (0.53)
Transverse series scales	13	11–13	11–13	12 (0)	12.13 (0.52)	12 (0)	12 (0.67)
Circumpeduncular rows scales[5]	17	14–17	13–16	16 (0)	15.87 (0.83)	14 (1)	13 (4.67)
Pectoral fin rays	12	12–13	12–13	12 (0)	12.2 (0.41)	12 (0.75)	12.3 (0.48)
Pelvic fin-rays	5	4–5	4–4	4 (1)	4.47 (0.52)	4 (0)	4 (0)
Dorsal fin-rays	26	23–29	18–22	26 (0)	25.87 (1.25)	21.5 (2)	20.9 (1.37)
Anal fin-rays	30	28–32	24–28	30 (2)	29.87 (1.36)	25.5 (1)	25.6 (1.26)
Caudal fin-rays	23	24–26	24–24	24 (1)	24.47 (0.92)	24 (0)	24 (0)

fin base on anterior and median portion; 3 rows of scales on caudal-fin base. Longitudinal series of scales 30–33, transversal series of scales 11–13, and circumpeduncular rows of scales 14–16 (two males with 17 and one female with 13), in both sexes. Males with 1 or 2 contact organs per scale on anterior central and ventral section of flanks and opercular region; rows of contact organs on 3–5 uppermost pectoral-fin rays and on anterior distal anal-fin rays; no contact organs on other fins and not present on females.

Cephalic neuromasts: supraorbital 16–18 (one with 15), parietal 1–3 (usually 3), anterior rostral 2–3 (usually 2 or 5 adding both sides of head), posterior rostral 1–2 (usually 1), infraorbital 2 + 22–27, preorbital 2 (one with 1), otic 1–3 (usually 2), post-otic 2–4 (usually 3–4), lateral supratemporal 1 (sometimes absent), median opercular 1–4 (usually 1), ventral opercular 3–5 (usually 3), preopercular 19–23 (usually 21–22),

mandibular 11–18 (usually 15–16), lateral mandibular 6–8, post-temporal 1–2 (usually 1). Lateral line complete, with one neuromast per scale.

Total number of vertebrae 30–32. Hypurals ankylosed in a single plate without visible fissure. Six branchiostegal rays, arranged in 3 pairs. Gill-rakers on 1st branchial arch 6–8 + 16–18.

Coloration in life. Males (Figs. 5–6). Head greenish gray, opercular region bluish gray to bluish dark green. Broad black bar crosses vertically the eye; subrectangular at suborbital portion, and subtriangular at supraocular portion rarely surpassing dorsally the line of parietal neuromasts. Iris yellow to dark yellow with black vertical bar through center of eye. Trunk and caudal peduncle ground color greenish gray, some paler at anteroventral region. Flanks and caudal peduncle sides with whitish to pale yellowish dots; sometimes, especially on anterior flanks, vertically elongated and usually vertically aligned in 5–11 unarranged rows. At anteromedial portion of flanks, approximately on center of linear axis between anal- and dorsal-fin bases, 1–2 vertically elongated black humeral blotches with size of eye diameter or larger, sometimes coalesced or unconspicuous. Pectoral fins hyaline to pale gray, with black margin at least ventrally, sometimes also distally and dorsally, and usually with a bluish gray iridescent band at ventral submargin. Pelvic fins pale gray to greenish gray, usually with black tip. Dorsal and anal fins greenish gray at basal half, usually with some whitish to yellowish spots following vertical alignment of spots on flanks; dorsal fin distal half hyaline to pale gray, sometimes posterodistal section dark gray to almost black and with black margin; anal fin distal half pale gray to greenish gray, submargin usually dark gray to almost black with black margin. Caudal fin basal half greenish to bluish gray, sometimes with metallic shine, and marginal section hyaline.

FIGURE 5 | *Austrolebias ayoreode*. Male and female (1 day after collection). Above: MNKP 16612, holotype, male, 50.7 mm SL, Bolivia, Santa Cruz, Cordillera, Charagua Iyambae Guarani Autonomous Territory, Ñembi Guasu Conservation and Ecological Importance Area. Below: MNKP 16587, paratype, female, 38.1 mm SL, collected with holotype.

Females (Figs. 5, 7). Head pale greenish gray with golden greenish iridescent postocular and opercular region. Iris yellow to dark yellowish with black vertical bar through center. Trunk and caudal peduncle ground color yellowish brown, greenish darker dorsally, on anteroventral and pectoral region pale yellowish turning whitish ventrally, sometimes golden iridescent on anterior flanks. Posterior flanks and caudal peduncle sides with dark grayish brown to pale black blotches irregularly distributed, turning inconspicuous with age. On anteromedial flanks, some anterior to the center of the linear axis between anal- and dorsal-fin bases, 1 to 4 black, sometimes greenish iridescent, vertical elongated humeral blotches. Fins hyaline; dorsal fin sometimes pale yellowish at basal half and with some black dots at posterior basal section; anal fin sometimes pale yellowish at anterior and median basal section and/or with some black dots at posterior basal section.

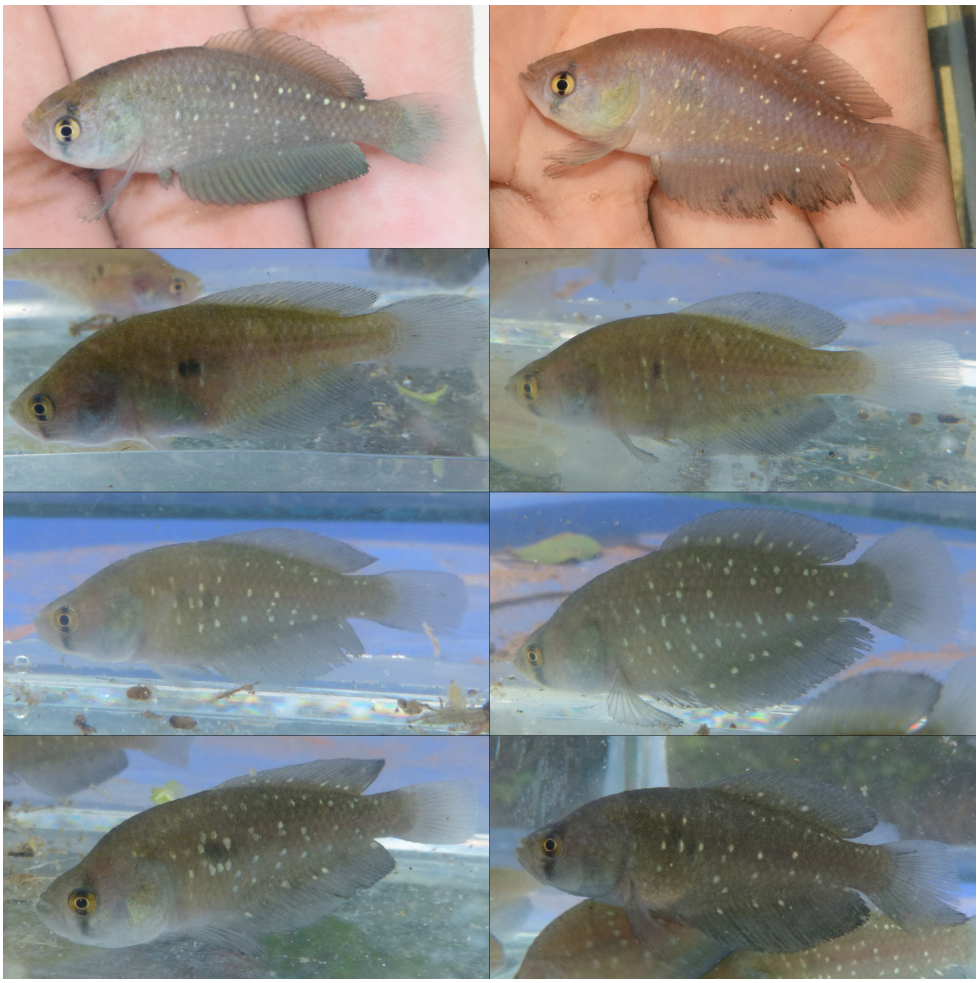

FIGURE 6 | *Austrolebias ayoreode*. Males (immediately after collection). Two uppermost: MNKP 16590, paratypes, 34.7–34.8 mm SL, Bolivia, Santa Cruz, Cordillera, Charagua Iyambae Guarani Autonomous Territory, Ñembi Guasu Conservation and Ecological Importance Area. Lower six: MNKP 16587, paratypes, 21.2–34.7 mm SL, collected with uppermost.

FIGURE 7 | *Austrolebias ayoreode*. Females (immediately after collection). Above: MNKP 16590, paratype, 26.1 mm SL; Bolivia, Santa Cruz, Cordillera, Charagua Iyambae Guarani Autonomous Territory, Ñembi Guasu Conservation and Ecological Importance Area. Below: MNKP 16587 (vertically flipped), paratype, female, 38.1 mm SL, collected with MNKP 16590.

Coloration in alcohol. Overall color pattern similar to that of live specimens, but less intense and faded, without iridescence and shines, or color hues. Body background color in males brown to grayish brown, in females yellowish gray to pale yellowish brown. Dorsal and anal fins in males dark gray to pale black.

Geographical distribution. This species is only known from two temporary pools in an unnamed watershed (HU 6424726) that drains its waters through an intermittent stream into the San Miguel River, part of the upper Paraguay River drainage in the La Plata basin (Fig. 3). The locality is situated in the Cordillera Province of the Santa Cruz Department in Bolivia, in close proximity to the Paraguayan border. With such a restricted known distribution, the species can be considered microendemic to the area.

Ecological notes. The species is found in two distinct types of aquatic ecosystems within the xeric woodlands of the Dry Chaco ecoregion, in the Chiquitano transitional to Chaco sector, locally known as "Abayoy". The first is a residual temporary pool of an intermittent watercourse with clear water (Fig. 8), not exceeding 30 cm in depth, featuring a thin layer of plant litter and debris (1 to 2 cm) over gray clay as substratum. It is situated in dense vegetation, with some low trees and shrubs growing in it, and includes submerged aquatic plants (*Echinodorus* sp., *Najas* sp., *Cabomba* sp. and *Riccia fluitans* Linnaeus, 1753), emergent species (mainly Poaceae species, and *Echinodorus* sp.) and abundant floating plants (*Nymphaea* sp., *Limnolobium* sp., *Lemna* sp., *Pistia stratiodes* Linnaeus, 1753, *Azolla* sp., and *Heteranthera* aff. *peduncularis*), which, in some well-illuminated areas, completely cover the water surface. The second aquatic ecosystem, located a few kilometers away from the first, is a temporary clearwater lagoon with a depth of up to 1.5 m and a detritus layer (*ca*. 5 cm) over sandy substrate. It exhibits a considerable diversity of aquatic vegetation, including submerged species (*Najas* sp., *Sagittaria* sp., *Echinodorus* sp., *Eleocharis* aff. *vivipara*, *Macaya* sp., and *R. fluitans*),

FIGURE 8 | Biotope at type-locality of *Austrolebias ayoreode*. Bolivia, Santa Cruz, Cordillera, Charagua Iyambae Guarani Autonomous Territory, Ñembi Guasu Conservation and Ecological Importance Area, 19°08'46"S 59°55'44"W, 10 Jun 2021. **A.** Well-illuminated area; **B.** Less illuminated area.

floating species (*P. stratiodes*, *Salvinia* sp., *Lemna* sp., *Limnolobium* sp., *Nymphoides* sp., and *Nymphaea* sp.), and emergent plants (Poaceae and Cyperaceae species, *Eleocharis* sp., *Echinodorus* sp., and *Sagittaria* sp.) in the riparian zone. This waterbody has extensions that extend into the surrounding shrubland, forming aquatic ecosystems with characteristics similar to those of the first described ecosystem where *A. ayoreode* inhabits. The only sympatric fish species in these ecosystems is *Neofundulus paraguayensis* (Eigenmann & Kennedy, 1903). The minimum annual temperature varies from 2.1 to 10.2°C (average 6.2°C), and the maximum ranges from 38.8 to 44.3°C (average 41.4°C), with minimums concentrated in the months of May to August and maximums from September to November. The accumulated annual precipitation can vary from 443 to 1334 mm, concentrating in the months of November to March, with a pronounced dry season between June and September.

Etymology. The name *ayoreode* is an eponym conferred in reference to the indigenous Ayoréode people, who historically occupied vast expanses of the northern Dry Chaco, and of whom a few groups still persist in the area of the type-locality of this species, standing as the last indigenous people in voluntary isolation outside of the Amazon in the Americas. Through this nomenclature, we intent to perpetuate the memory of the existence of the Ayoréode and emphasize the imperative of preserving their ancestral territory, which also is the habitat of the species described herein.

Conservation status. The species is known only from two closely situated (approximately 4 km) and isolated temporary water bodies in xeric woodlands and shrublands. Despite intensive sampling on multiple occasions in the area, the species was not found outside these specific waterbodies. Considering this, the estimated area of occupancy (AOO) is challenging due to the scarcity of small ephemeral and temporary water bodies in the xeric ecosystems of the Chiquitano transitional to Chaco sector near the border between Bolivia and Paraguay; detection and quantification with telemetric methods are difficult. Based on the currently known occurrences, the species has an AOO of less than 10 km², but this estimate has a high degree of imprecision, and the AOO could potentially be higher, although unlikely to exceed 500 km². Despite the entire AOO being within a subnational conservation unit (Ñembi Guasu Conservation and Ecological Importance Area), this does not exempt the species from threats to its conservation status and does not guarantee the stability of its population. On one hand, the legal security in Bolivia of subnational conservation areas, such as Ñembi Guasu, is fragile and uncertain, while, on the other hand, there is strong social demand for productive use of these territories. Additionally, the direct and indirect effects of climate change pose serious threats to both this species and the ecosystems it inhabits. Changes in hydrological regimes, such as prolonged and/or intense drought periods, can negatively affect the embryonic and/or ontogenetic development of species subpopulations. Indirectly, the increased incidence of wildfires, similar to those that occurred in the area in 2019, reaching sixth-generation levels and triggering firestorms, can rapidly degrade the habitat quality for these fish. Following the guidelines for categories and criteria of the IUCN, the species may be categorized as Endangered (EN) as it meets the criteria B2a,b(ii,iii) (IUCN Standards and Petitions Committee, 2022).

DISCUSSION

Both species, *Austrolebias accorsii* and *A. ayoreode*, meet the diagnostic characters established for the genus by Alonso *et al*. (2023), except for the urogenital papilla in males. In *A. ayoreode* the urogenital papilla is mostly attached to the anal fin, although this fusion is incomplete and only reaches about 70–80% of the basal portion. In contrast, in *A. accorsii* it is only attached basally, and the fusion barely reaches approximately 10% of the basal portion, very similar to how it is also in males of *A. vandenbergi*. There are also subtler characteristics than those presented in the diagnosis, or with partially overlapping ranges in the case of morphometric and meristic values, which in some instances may be useful for differentiating *A. accorsii* and *A. ayoreode* from the remaining species in the genus, especially those morphologically more similar.

Thus, males of *A. accorsii* can often be distinguished from those of *A. bellottii*, *A. ephemerus*, and *A. univentripinnis* by having more longitudinal series of scales (30–34 *vs*. 28–32, 27–30, 28–30, respectively) and transversal series than *A. ephemerus*, *A. melanoorus*, *A. queguay*, and *A. univentripinnis* (14–18 *vs*. 13–14, 12–15, 11–16, 13–14, respectively); from *A. ephemerus*, *A. univentripinnis*, and *A. vandenbergi* by dorsal-fin origin on vertical between 4th and 6th anal-fin ray (*vs*. between vertical on 1st and 4th anal-fin ray); from *A. ephemerus* by having shorter pectoral fins (18.8–25.6% SL *vs*. 22.4–27.1%) and greater head width (58.2–75.6% HL *vs*. 55.3–61.1%); from *A. melanoorus* by having more anal-fin rays (28–36 *vs*. 25–28); and from *A. vandenbergi* by having a smaller size (max. SL 55.2 mm *vs*. 79.7 mm), lower body depth (35.0–43.9% SL *vs*. 39.6–46.7%), shorter dorsal-fin base (35.0–43.4% SL *vs*. 38.0–47.0%), longer pelvic fins (8.6–13.0% SL *vs*. 7.7–9.5%), lower head depth (102.1–124.8% HL *vs*. 110.4–136.9%), more dorsal-fin rays (22–32 *vs*. 18–28), more anal-fin rays (28–36 *vs*. 24–32), and more posterior dorsal-fin origin (vertical between 4th and 6th anal-fin ray *vs*. between 2nd and 5th). Furthermore, the presence of whitish spots on the flanks of males can serve as a distinguishing character, as these spots are occasionally observed in *A. vandenbergi*, but are absent in *A. accorsii*. Females of *A. accorsii* usually can be distinguished from those of *A. bellottii, A. ephemerus*, *A. melanoorus*, and *A. queguay* by having more dorsal-fin rays (20–27 *vs*. 17–22, 18–20, 17–21, 17–20, respectively); from those of *A. melanoorus* and *A. univentripinnis* by having more anal-fin rays (25–35 *vs*. 21–25, 22–26, respectively); and from *A. ayoreode* and *A. ephemerus* by dorsal-fin origin on vertical between 5th and 10th anal-fin ray (*vs*. between 4th and 5th, or 2nd and 5th, respectively).

Austrolebias ayoreode can normally be distinguished from all congeners by the presence of one or two dark vertically elongated spots on anteromedial flanks of males, but this character can be inconspicuous in some males and may not always be visible, and it is also present in some males of *A. bellottii*. Males of the new species also usually differ from those of *A. bellottii*, *A. ephemerus*, *A. melanoorus*, and *A. univentripinis* by having a shorter predorsal length (45.3–54.3% SL *vs.* 49.7–57.7%, 50.1–52.1%, 48.8–55.5%, 49.5–52.9%, respectively) and by less transversal series of scales (11–13 *vs*. 12–17, 13–14, 12–15, 13–14, respectively); from *A. accorsii*, *A. bellottii*, and *A. vandenbergi* by a shorter anal-fin base (39.0–45.9% SL *vs*. 35.6–52.9%, 44.9–54.8%, 46.3–51.1% [40.1–51.1% on comparative material, Tab. **S1**], respectively); from *A. accorsii*, *A. bellottii, A. ephemerus*, and *A. melanoorus* by having a lower body depth (32.6–38.6% SL *vs*. 35.0–43.9%, 38.0–46.1%, 34.0–40.9%, 36.6–41.3%, respectively); from *A. bellottii*, *A. ephemerus*, and

A. univentripinnis by more scales in logitudinal series (30–33 *vs*. 28–32, 27–30, 28–30, respectively); from *A. ephemerus*, *A. univentripinnis*, and *A. vandenbergi* by dorsal-fin origin on vertical between 4th and 5th anal-fin ray (*vs*. between 1st and 4th or 2nd and 4th for the last two); from *A. bellottii*, *A. univentripinnis*, and *A. vandenbergi* by a lower head depth (100.1–117.7 % SL *vs*. 110.3–128.5%, 109.6–118.8%, 110.4–121.3%, respectively); from *A. melanoorus*, *A. univentripinnis*, and *A. vandenbergi* by a shorter dorsal-fin base length (33.7–44.3% SL *vs*. 37.7–46.7%, 40.1–44.4%, 38.0–42.5% [40.3–47.6% on comparative material examined, Tab. **S1**], respectively), and from *A. ephemerus* by a longer dorsal-fin base (*vs*. 35.6–41.5%); from *A. accorsii* and *A. ephemerus* by having less anal-fin rays (28–32 *vs*. 28–36, 27–30, respectively), and from *A. melanoorus* by more anal-fin rays (*vs*. 25–28); from *A. bellottii* and *A. ephemerus* by tip of pelvic fins reaching between 1st and 2nd anal-fin ray (*vs*. between 2nd and 5th anal-fin ray); from *A. ephemerus* and *A. queguay* by having more dorsal-fin rays (23–29 *vs*. 24–25, 22–25, respectively); from *A. accorsii* by a wider head width (65.0–83.5% SL *vs*. 58.2–75.5%); from *A. ephemerus* by larger size (max. SL 50.7 mm *vs*. 31.7 mm) and tip of pectoral fin reaching between 4th and 6th anal-fin ray (*vs*. between 6th and 8th anal-fin ray); and from *A. queguay* by a shorter prepelvic length (38.4–46.7% SL *vs*. 41.3–48.8%). Females of *A. ayoreode* can also be distinguished from those of *A. bellottii*, *A. univentripinnis*, and *A. vandenbergi* by a lower body depth (33.4–38.0% SL *vs*. 35.4–45.5%, 36.0–40.4%, 37.2–41.1%, respectively); from *A. accorsii* and *A. ephemerus* by having shorter anal-fin base length (23.7–29.9% SL *vs*. 27.9–40.2%, 28.4–32.4%, respectively), and shorter head length (26.5–31.8% SL *vs*. 27.9–34.8%, 30.6–33.7%, respectively); from *A. ephemerus* and *A. vandenbergi* by a shorter pre-dorsal length (49.0–58.1% SL *vs*. 55.5–62.5%, 57.1–61.3%, respectively); from *A. accorsii* by having less dorsal-fin rays (18–22 *vs*. 20–27) and anal-fin rays (24–28 *vs*. 25–35); and from *A. ephemerus* by tip of pelvic fin reaching between 1st and 2nd anal-fin ray (*vs*. between 2nd and 4th anal-fin ray). The presence of dark dots on the posterior section of the dorsal and anal fins of some females of the new species could also be used to distinguish it, as this character is not observed in females of other species within the genus.

Within *Austrolebias*, two species groups can be differentiated based on morphological, geographical, and ecological characteristics: herein named the *A. melanoorus* group and the *A. vandenbergi* species group. Both *A. accorsii* and *A. ayoreode* are included in the *A. vandenbergi* species group, to which also belongs *A. ephemerus*. These species are primarily characterized by the presence of multiple rows of scales that extend beyond the base of the anal fin, at least in the anterior section, in both males and females; additionally, males of this species group have contact organs on the distal portion of the anterior rays of the anal fin [occasionally also present in *A. bellottii* according to Costa (2006)] (Figs. **S2–S3**). They are distributed in the northern area of the Chacoan province (Fig. 9) in northern Argentina, southeastern Bolivia, southwestern Brazil (Mato Grosso do Sul), and northern Paraguay; in the upper Paraguay River basin, except for *A. accorsii*, which is found in the adjacent upper Iténez/Guaporé River basin. These species inhabit temporary pools, generally surrounded by shrublands or woodlands, which fill up between September and December, hold water during the summer, and dry out during the winter (Huber, 1995; Alonso *et al*., 2016; Volcan, Severo-Neto, 2019; Alonso, 2022).

The *Austrolebias melanoorus* species group, which also includes *A. bellottii*, *A. queguay*, and *A. univentripinnis*, is characterized by males having the urogenital papilla attached to the anal fin. Although this character is also present in *A. ayoreode* and *A. ephemerus*

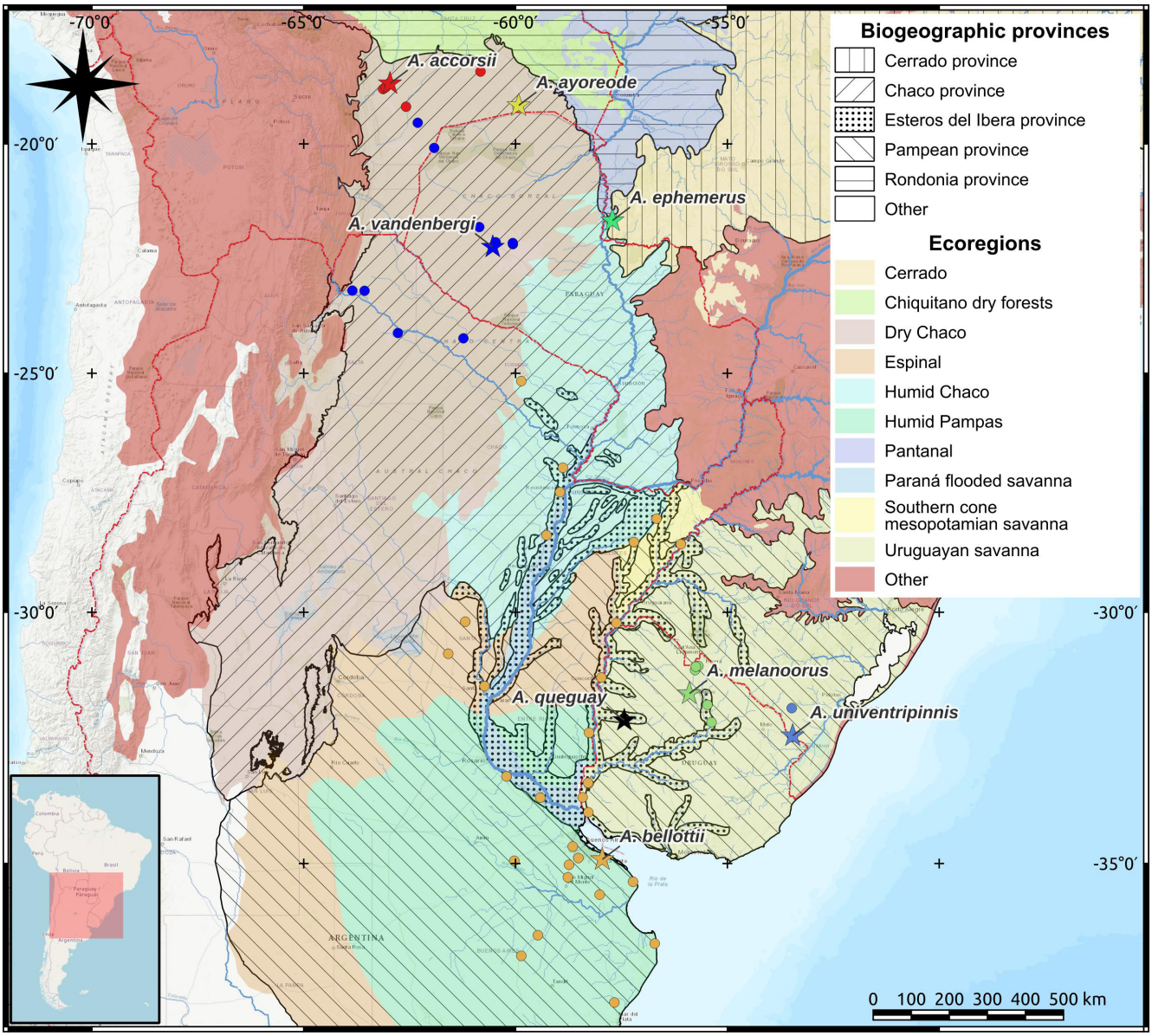

FIGURE 9 | Occurrence localities of *Austrolebias* species. Stars represent type-localities, dots other records. Colors differentiate the species (*A. accorsii* red, *A. ayoreode* yellow, *A. bellottii* dark yellow, *A. ephemerus* green, *A. melanoorus* pale green, *A. queguay* black, *A. univentripinnis* pale blue, *A. vandenbergi* blue) Sources: Biogeographic provinces from Morrone *et al.* (2022); Ecoregions from Olson *et al.* (2001); Basemap ESRI World Topo (2022).

(Volcan, Severo-Neto, 2019), in these species of the *A. vandenbergi* species group, the fusion is less extensive (only around 75% of the urogenital papilla) and the urogenital papilla is somewhat more separated from the first anal-fin ray, making it a partial and less complete fusion than in species of the *A. melanoorus* species group. This species are distributed in the Pampean and Esteros del Ibera provinces (Fig. 9) in central Argentina, southern Brazil, and Uruguay; in the basins of the rivers Paraná, Uruguay, lower La Plata, and the hydrologic system of Laguna Merín. As an exception, *A. bellottii* is also reported from a few locations in the lower Paraguay River basin, near its confluence with the Paraná River, in the Chacoan province in the southern Humid Chaco ecoregion

(Alonso *et al*., 2020). They inhabit temporary pools mainly in grasslands that fill up in winter and remain dry during the summer months (Alonso *et al*., 2016; Loureiro *et al*., 2016; Volcan *et al*., 2016; Serra, Loureiro, 2018).

Austrolebias accorsii is the only species of the genus with distribution in the Amazon macrobasin (Nielsen, Pillet, 2015a; Alonso *et al*., 2016), in the region of the Bañados del Izozog wetlands. The Bañados del Izozog are fed by the Parapetí River, which originates in the eastern Andes slope and disperses its waters upon entering the Chacoan plain, forming the wetland. These waters are collected by watersheds of several tributary rivers of the San Julián River, downstream called San Pablo River and then Itonamas River, before pouring its waters into the Iténez/Guaporé River near the town of Costa Marques (Rondônia, Brazil). The presence of *A. accorsii* in the upper Iténez/Guaporé River basin is explained by the fact that the Parapetí River formerly belonged to the Paraguay River basin, and only during the late Miocene, approximately 10–8 million years ago, did it gradually change its course northward, draining into the Iténez/Guaporé River (Lundberg *et al*., 1998; Navarro, Maldonado, 2002; Costa, 2010). On the other hand, the presence of *A. accorsii* in the Mamoré River basin has not been confirmed so far, as all references are based on the type-locality indicated by Nielsen, Pillet (2015a) incorrectly located in the Grande/Guapay River basin, probably due to its proximity to this river, and not in the Parapetí River basin where it is actually located. Likewise, the presence of *A. vandenbergi* in the Parapetí River basin reported by Montaña *et al*. (2012) is dismissed, as the specimens presented by them (Montaña *et al*., 2012: fig. 1) clearly belong to *A. accorsii* considering the diagnostic characters presented in the present species redescription. Consequently, the study on reproductive strategies of *A. vandenbergi* conducted by Schalk *et al*. (2014) actually corresponds to *A. accorsii* since it is based on the same material (TCWC 15182.01, TCWC 15183.01, and TCWC 15184.01) cited by Montaña *et al*. (2012).

For the interpretation of the central tendency and measure of dispersion of morphometric and meristic data in fishes, the average and standard deviation are usually used, as is the case in practically all descriptions and redescriptions of rivulids until now. However, these data mostly exhibit a non-Gaussian distribution (lack of statistical normality), which can be tested with skewness and kurtosis analysis (Hopkins, Weeks, 1990; McCluskey, Lalkhen, 2007; Guthrie, 2020). Therefore, neither the average is the ideal statistical parameter to summarize the central tendency of the data, nor is the standard deviation appropriate to show the degree of dispersion of the data (Hopkins, Weeks, 1990; McCluskey, Lalkhen, 2007; Szoszkiewicz *et al*., 2010). The main problem of using the average (mean) as the measure of central tendency in this case lies in its sensitivity to extreme or atypical values (outliers), which are quite common in morphometric and meristic data of rivulids. Consequently, the use of the median as measure of central tendency and the interquartile range (IQR) as measure of dispersion of values is a more suitable alternative considering the characteristics of the data, the simplicity of its interpretation and calculation, and the robustness against outliers. The median will show us the typical value of the morphometric or meristic character, from which half of the examined specimens will have a lower value and the other half a higher value for the given character; the IQR will indicate how close or far, in the same unit of measure, the values of half of the examined specimens are distributed around the median value.

Comparative material examined. *Austrolebias bellottii*: **Argentina:** NMW 75105 (photo only), male (47.5 mm SL), holotype. MLP 3643 (photo only), 2 males (39.5–49 mm SL). UFRJ 4743 (photo only), male (44.8 mm SL), female (33.7 mm SL). **Uruguay:** MHNM 3216 (photo only), male (41.8 mm SL). UFRJ 6226 (photo only), female (32.9 mm SL). ZVC-P 11560 (photo only), female (not measured). *Austrolebias ephemerus*: **Brazil:** ZUFMS 4144 (photo only), male (22.2 mm SL). ZUFMS 5464, female (28.1 mm SL), paratype. ZUFMS 5465 (photo only), male (28.6 mm SL), holotype. ZUFMS 5725 (photo only), male (29.1 mm SL), female (26.6 mm SL), paratypes. *Austrolebias melanoorus*: **Uruguay:** UFRJ 6161 (photo only), male (30.1 mm SL), female (30.3 mm SL). ZVC-P 13651 (photo only), male and female (not measured), topotype. *Austrolebias queguay*: **Uruguay:** ZVC-P 11620 (photo only), male and female (not measured), paratypes. ZVC-P 13576 (photo only), male (39.4 mm SL), holotype. *Austrolebias univentripinnis*: **Brazil:** UFRGS 18064 (photo only), male (not measured). UFRGS 18066 (photo only), female (not measured). UFRJ 6081 (photo only), male (34.9 mm SL), holotype. UFRJ 6082 (photo only), female (32.2 mm SL), paratype. **Uruguay:** MHNM 4627, 6 males (33.9–44.2 mm SL), 4 females (31.3–34.2 mm SL). *Austrolebias vandenbergi*: **Argentina:** MACN-Ict 8180 (photo only), male (49 mm SL). MACN 9701 (photos only). MACN 9707 (photos only). MHNM 2575 (photo only), male (69.1 mm SL). **Bolivia:** MNKP 3288, 2 males (35.5–37.4 mm SL). MNKP 3290, 5 males (56.7–75 mm SL), 2 females (50.7–52.2 mm SL). MNKP 3293, 5 males (65.3–79.7 mm SL), 6 females (49.7–67.2 mm SL). MNKP 3294, 2 males (39.8–51.5 mm SL), 3 females (39.8–56.5 mm SL). **Paraguay:** ANSP 175282 (photos only), 5 males (36.7–48.1 mm SL), 11 females (28.4–39.7 mm SL), topotypes. ANSP 175283 (photos only), 3 males (33.3–41.3 mm SL). MHNM 2557 (photo only), 1 male (46.8 mm SL), 6 females (25.0–52.3 mm SL). UFRJ 3028 (photo only), male (63.8 mm SL), paratype.

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AUTHORS' CONTRIBUTION

Heinz Arno Drawert: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

Carlos Ergueta: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing-review and editing.

ETHICAL STATEMENT

The collection of materials for this study was conducted with the authorization of the Ministry of Environment and Water of Bolivia (MMAyA) through the General Directorate of Biodiversity and Protected Areas (DGBAP; Authorization: CAR/MMAYA/VMABCCGDF/DGBAP/MEG #0455/2021).

COMPETING INTERESTS

The author declares no competing interests.

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