

THE RISE OF INTERNAL FERTILIZATION IN THE ANABLEPIDAE (TELEOSTEI, CYPRINODONTIFORMES): TWO NEW GENERA AND SPECIES FROM THE MIOCENE OF TUCUMÁN, ARGENTINA

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Abstract: Two new genera and species of Cyprinodontiformes from the Miocene of Tucumán, Argentina, are described: *Tucmanableps cionei* and *Sachajenymsia pacha*. Both exhibit sexual dimorphism, indicated by the modification of the male anal fin into an intromittent organ; tricuspid teeth in the jaws; abdominal pelvic fins and other features that demonstrate a relationship with the family Anablepidae. Males of *Sachajenymsia pacha* gen. et sp. nov. present modifications of the anal fin, herein interpreted as an incipient gonopodium, whereas the anal fin of *Tucmanableps cionei* gen. et sp. nov. is modified into a complex gonopodium with a median distal plate, which is unique among the Anablepidae. In extant genera of the family, modification of the anal fin into an intromittent organ is related to internal fertilization; this condition is inferred for *S. pacha* but was clearly the reproductive mode of *T. cionei*. In our

phylogenetic analysis, *S. pacha* is recovered as the sister group of all anablepids with evident internal fertilization, while *T. cionei* forms a clade with *Anableps* species. The occurrence of two new anablepid genera showing distinct and unique morphologies suggests that the fossil diversity of the Anablepidae may be higher than the extant one. Also, the new taxa were found in open lake deposits with coastal and inner lake sediments of the Río Salí Formation associated with the Miocene Entrerrián–Paranense Marine Ingression, supporting the idea that coastal environments and brackish waters are related to the generic diversification of the Anablepidae, as was suggested for the species of *Jenymsia*.

Key words: internal fertilization, Teleostei, gonopodium, phylogeny, Miocene Entrerrián–Paranense Marine Ingression, Anablepidae.

THE order Cyprinodontiformes constitutes the most diversified group of atherinomorph fishes, including about 1120 species distributed in 125 genera (Nelson 2006; Costa 2012). Cyprinodontiform fishes are found in subtropical and tropical areas of Asia, Europe, Africa and Americas, occurring commonly in freshwater and brackish environments and less frequently in coastal marine environments (Costa 1998, 2012; Nelson 2006; Vila *et al.* 2011; Calviño & Alonso 2016).

In South America, the order is represented by the families Cyprinodontidae, with 45 species, Rivulidae, with 421 species, Poeciliidae, with 66 species, and Anablepidae, with 16 species. This order is an important component of the biota from continental aquatic environments of most Neotropical basins, including those from north and central Argentina (Ghedotti 2003; Lucinda 2003; Calviño

2005; Aguilera & Mirande 2005; Liotta 2006; Nelson 2006; Aguilera *et al.* 2009; Mirande & Koerber 2015; Eschmeyer *et al.* 2017).

Even though at present the order is highly diverse, its fossil record is comparatively scarce. To date, only one fossil species of the suborder Aplocheiloidei has been reported (i.e. *Kenyaichthys kipkechi* Altner & Reichenbacher, 2015, from the Miocene of Kenya) whereas all remaining known fossil cyprinodontiforms are members of the suborder Cyprinodontoidei and are almost restricted to the northern hemisphere. These include two *incertae sedis* cyprinodontoid genera probably related to the Valenciidae (*Prolebias* Sauvage, from the Oligocene and Miocene of Europe and Asia, and *Aphanolebias* Reichenbacher & Gaudant, from the Miocene of Europe; Costa 2012), a few fossil species of extant genera of

Cyprinodontidae (Miocene and Pliocene of Europe, USA and Asia; Smith 1981; Gaudant 1993; Vasilyan *et al.* 2009), Fundulidae (early–middle Miocene to middle Pliocene of USA; Ghedotti & Davis 2017), Poeciliidae (Pleistocene of Mexico; Guzmán & Polaco 2009) and Goodeidae represented by the fossil genus *Tapatia* Álvarez & Arriola-Longoria, from the late Miocene to late Pliocene of North America (Altner & Reichenbacher 2015 and references cited therein) and *Empetrichthys erdisi* (Jordan 1924) from the middle Pliocene of USA (Uyeno & Miller 1962).

In South America, with the exception of *Carrionellus diumortuus* White, 1927, from the early Miocene of Ecuador (considered to be an anablepid by Ghedotti 1998 and later reassigned to the Cyprinodontidae by Costa 2011) and *Jenynsia* sp. Günther, from the Pleistocene of Argentina (Bogan *et al.* 2009), the record of fossil cyprinodontiforms is poorly known. It includes mentions of undetermined Cyprinodontiformes from Paleocene and Eocene sediments of Argentina, as well as the Neogene of Brazil (Parenti 1981; Cione 1986; Arratia & Cione 1996; Cione & Báez 2007), probable anablepids in the San José Formation (late Miocene) in Catamarca and Tucumán and probable poeciliids in the Río Salí Formation (middle Miocene) in Tucumán, Argentina (Bossi & Palma 1982; Gavrilloff & Bossi 1992; Arratia & Cione 1996; Anzotegui & Herbst 2004; Cione & Báez 2007).

Poeciliines and anablepines are very peculiar cyprinodontiforms because they have internal fertilization and all except for one species (*Tomeurus gracilis* Eigenmann, 1909) are viviparous fishes. Members of these families exhibit a profound sexual dimorphism as the male anal fin is modified into an intromittent organ or gonopodium (Parenti 1981). Contrary to poeciliines, in which the gonopodium is formed mainly by three anal-fin rays, in the anablepines it is formed by all the anal-fin rays crowding together and twisting around each other in *Anableps* Scopoli and by anal-fin rays three and five to seven in *Jenynsia* (Parenti 1981).

In particular, the middle Miocene remains from the Río Salí Fm. of Tucumán, in north-western Argentina, consist mostly of well-preserved, complete and articulated cyprinodontiforms that were previously assigned to Poeciliidae (Cione & Báez 2007). However, according to our observations on the position of fins, type of teeth in the premaxilla as well as the gonopodium configuration of males (they exhibit sexual dimorphism), and our phylogenetic analysis, these fishes correspond to two new genera and species of Anablepidae.

At present, this family is composed of three morphologically distinct genera, the monotypic *Oxyzygonectes* Fowler, *Jenynsia* (with 13 species) and *Anableps* (with three species). The occurrence of two new different anablepid genera in the Miocene of Tucumán, Argentina

shows that the diversity of the family might have been larger in the past, involving highly divergent taxa with distinct and unique morphologies and already showing a sexual reproduction by means of internal fertilization. A description of the new taxa and a discussion on their phylogenetic position is provided here.

GEOLOGICAL SETTING

The Río Salí Formation (Fig. 1) of middle Miocene age is the lowermost stratigraphic unit of the Choromoro Group, Valle del Choromoro, province of Tucumán, Argentina, which also includes the India Muerta Formation (Bossi *et al.* 1998). The Río Salí Formation was first described by Ruiz Huidobro in 1960 and later redefined by Bossi (1969) who recognized three informal members within the unit: from base to top Salina López, Aliviadero and Tapia. The studied fish material comes from the basal Salina López member, which also housed bivalves, insects and ostracods (Bossi *et al.* 1998). The Salina López member is composed of fine to medium grained sandstones with cross-stratified strata which grade upwards to greenish and yellowish argillites and siltstones, and marls finely interbedded with bituminous siltstones and also with nodules of gypsum (Bossi 1969). In general, the sedimentary succession is interpreted as an open lake with coastal facies and inner lake facies (Bossi *et al.* 1998), which have been associated with the Entrerriean–Paranense Marine Ingression (Georgieff *et al.* 2014), although the western limits of the ingression are not clearly known (Herbst *et al.* 2000).

The Río Salí Formation is partially correlated with the San José Formation of the Neogene Santa María Group (Fig. 1) of late Miocene to Pliocene age (Gavrilloff & Bossi 1992). However, the Santa María Group is not strictly related; it is totally continental, as shown by its freshwater (eventually slightly brackish) fauna (ostracods, bivalves and gastropods) as well as the megaf flora (leaves and fruits, Anzotegui & Herbst 2004). The Santa María Group also contains fossil cyprinodontiforms in the San José and Chiquimil formations, which are quite different to those described herein and are currently being studied by the authors.

The specimens of *Sachajenynsia pacha* were all collected in 2007 in the locality of Toma de Vipos (26° 29' 56" S, 65° 23' 55" W; Fig. 1, locality 1). This outcrop shows a succession of about 30 m of intercalated tabular, laminated strata of yellowish and greenish argillites and siltstones to fine-grained sandstones with rippled to parallel lamination; and carbonate to marl levels with interbedded levels of gypsum. A horizon rich in nodules of gypsum characterized the upper levels. The occurrence of *S. pacha* is in the marl levels just below this horizon.

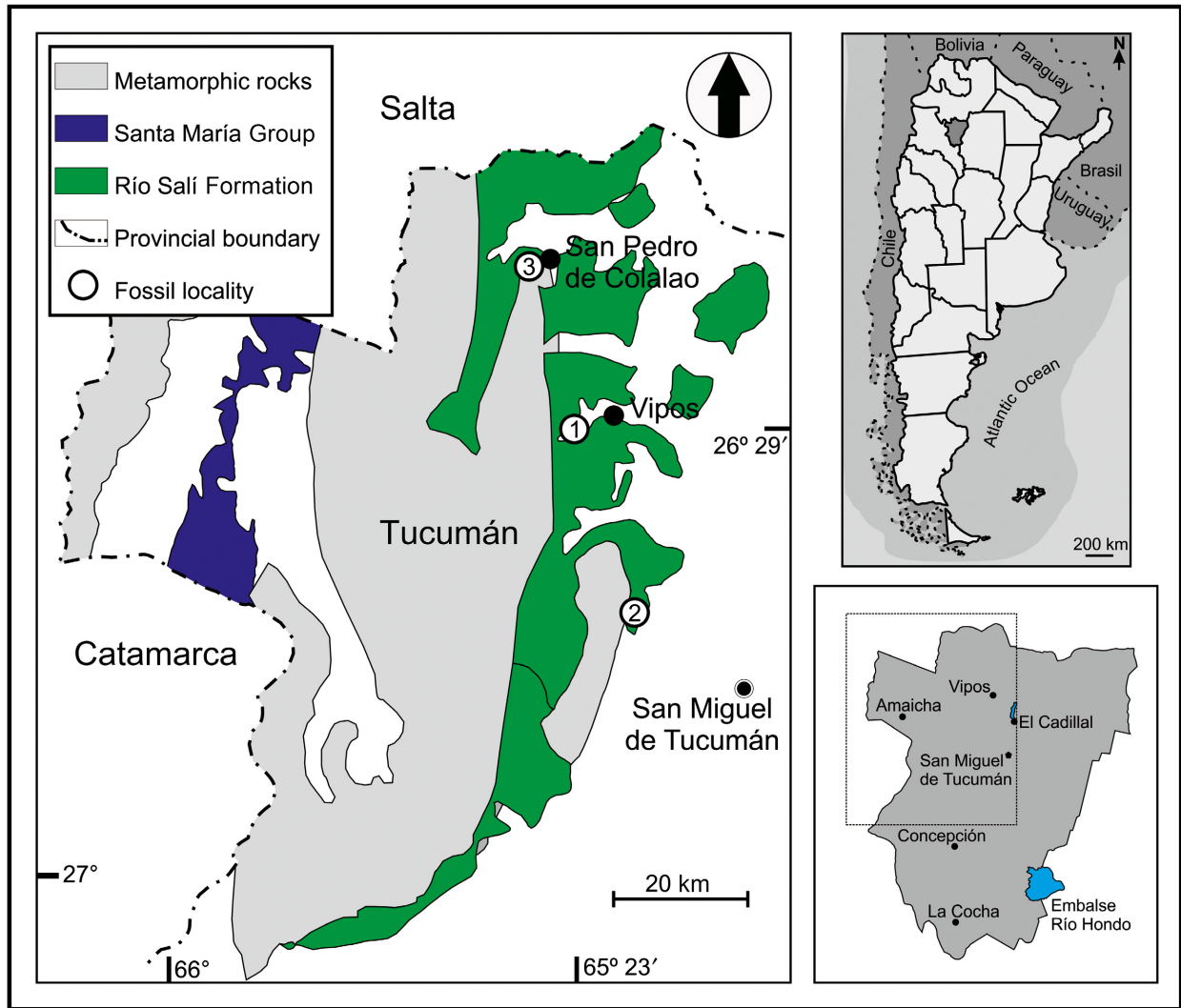


FIG. 1. Geological map of the north-western region of Tucumán province, Argentina. Numbers indicate the collecting localities of *Sachajenynsia pacha* (1) and *Tucmanableps cionei* (2–3) in the Río Salí Formation. 1, Toma de Vipos; 2, Valle de San Javier; 3, Arroyo Angostura.

The specimens of *Tucmanableps cionei* were collected in several exploratory fieldtrips between 1950 and 1960 from two localities: Valle de San Javier and Arroyo Angostura (Fig. 1, localities 2 and 3, respectively). Although the exact stratigraphical position within the Río Salí Formation is unknown (unfortunately it was not specified in the collection notes), fishes occur in levels of marls that are always found in the lower part of the Salina López member (Bossi *et al.* 1998). Specimens PVL 2398, 2410–2412 and 2414 were found in outcrops of the Río Salí Formation in the Valle de San Javier, at the side of the road on the way to El Siambón, provincial route RP340, approximately 30 km from San Miguel de Tucumán (26° 39' 35.70" S, 65° 23' 33.16" W). On the other hand, specimens PVL 3579, 3639–3641 come from outcrops at the Arroyo

Angostura, located west of San Pedro de Colalao, province of Tucumán (26° 14' 44.98" S, 65° 32' 14.33" W).

MATERIAL AND METHOD

The fossil fish material studied here is deposited in the Vertebrate Palaeontology Collection of the Instituto-Fundación Miguel Lillo in Tucumán, Argentina (PVL). Fish specimens were mechanically prepared at the Fundación Miguel Lillo and the palaeontological laboratory of the CICTERRA and studied using binocular microscopes. Drawings were made with camera lucida and digitized with illustration software (Adobe Photoshop CS3 and Adobe Illustrator CS3). Measurements are straight distances between two points, taken with calipers to the

TABLE 1. Measurements of *Tucmanableps cionei*.

Collection number	TL	SL	BD	HL	PDL	PAL
PVL 2412(m)	23.46	18.64	16.2	25.6	66.7	56.7
PVL 2398(f)	31.83	25.38	15.1	27.3	67.8	61.2
PVL 2410(f)	23.50	18.78	20.5	27.5	69.4	61
PVL 2411(f)	24.74	19.83	16.6	31.6	69.1	63.2
PVL 3579(m)	14.54	12.49	20.4	23.2	66.6	61.2
PVL 3639(f)	28.99	22.77	16.6	29.7	70.9	62.8
PVL 3641(f)	25.68	21.92	12.7	28	70.2	62.5

BD, body depth; HL, head length; PAL, preanal length; PDL, predorsal length; SL, standard length; TL, total length. TL and SL expressed in mm, BD, HL, PDL and PAL expressed as percentages of standard length. Holotype in **bold**; f, female; m, male.

nearest 0.02 mm and are expressed as percentages of the standard length (SL) (Tables 1, 2). Measurements taken were: total length (TL, from snout tip to posterior border of the caudal fin); standard length (SL, from the tip of the snout to the posterior border of the hypural complex); body depth (BD, maximum depth of the body); head length (HL, from the tip of the snout to the posterior border of the opercle); preanal length (PAL, from the tip of the snout to the anal-fin origin); predorsal length (PDL, from the tip of the snout to the dorsal-fin origin). Skull bones are named according to Parenti (1981) and subsequent authors (e.g. Ghedotti 1998, 2000; Lucinda *et al.* 2002; Aguilera *et al.* 2013).

Anatomical abbreviations. angart, anguloarticular; a.p, anal-fin pterygiophores; a.r, anal-fin rays; bp, basipterygium; br, branchiostegal rays; cl, cleithrum; co, coracoid; c.r, caudal-fin rays; de, dentary; d.p, dorsal-fin pterygiophores; d.pl, distal plate; d.r, dorsal-fin rays; ep, epural; epi, epipleural; epo, epioccipital; ex.ha, expanded haemal spines; fr, frontal; ha, haemal spines; hyo, hyomandibular; hy.p, hypural plate; iop, interopercle; mx, maxilla; n, neural spine; op, opercle; p, parasphenoid; pa, parietal; pal, palatine; pap, parapophysis; pec.r, pectoral-fin rays; pel.r, pelvic-fin rays; ph, parhypural; pmx,

TABLE 2. Measurements of *Sachajenymsia pacha*.

Collection number	TL	SL	BD	HL	PDL	PAL
PVL 7036 (m)	36.6	32.8	17	27.4	65.2	58.2
PVL 7037 (f)	42.4	37.6	20.2	26.5	64	61.1
PVL 7038 (u)	34.7	30.4	—	—	62.7	54.6
PVL 7040 (m)	—	37.1	18	26.6	62.8	58.4
PVL 7041 (f)	47.1	41.3	16.4	24.6	64.8	62.2

BD, body depth; HL, head length; PAL, preanal length; PDL, predorsal length; SL, standard length; TL, total length. TL and SL expressed in mm, BD, HL, PDL and PAL expressed as percentages of standard length. Holotype in **bold**; f, female; m, male; u, undetermined.

premaxilla; pop, preopercle; pro, prootic; pte, pterotic; ptt, posttemporal; q, quadrate; r, ribs; ra, radial; re, retroarticular; sc, scapula; scl, supracleithrum; soc, supraoccipital; sop, subopercle; sph, sphenotic; st, stegural; sy, symplectic; tr.te, tricuspid teeth; v.pcl, ventral postcleithrum. The labels '(r)' or '(l)' after any of the abbreviations indicate right or left elements respectively.

Comparative material

Extant taxa examined for comparison in this work included cleared and stained as well as alcohol-preserved specimens of *Jenynsia alternimaculata*, *J. lineata*, *J. luxata*, *J. maculata*, *J. multidentata*, *J. obscura*, *J. tucumana*, *J. sanctaecatarinae*, *J. unitaenia* and *J. eirmostigma*.

Phylogenetic analysis

The new taxa were included in the character–taxon matrix of Ghedotti (1998), with the modifications proposed by Aguilera & Mirande (2005) and Aguilera *et al.* (2013), to evaluate their position within the Anablepidae. The data matrix for the present study is available in MorphoBank (Sferco *et al.* 2017). The complete dataset is composed of 71 characters and 24 species, including the outgroups. Analyses were rooted on *Profundulus labialis*. Among the studied material, there are well-preserved male and female specimens for both new taxa and the analysed dataset is mostly osteological. This allowed us to code as much of 54% of the characters in *Tucmanableps cionei* and 44% in *Sachajenymsia pacha*. Given that *S. pacha* lacks an evident gonopodium, the characters referred to it were coded as inapplicable to this taxon. Except for the characters 19, 30, 40, 46 and 58, which were considered as ordered, all remaining characters were analysed as unordered in the dataset from Ghedotti (1998). As in previous papers, the dataset from Ghedotti (1998) was analysed without constraints.

Cladistic parsimony analyses were performed with TNT (Goloboff *et al.* 2003a) under implied weighting (Goloboff 1993), which was designed to down-weight characters according to their homoplasy. Several concavity constants (*K* values) were used to assess differences in the relationships of the new taxa. We also performed analyses under equal weighting in order to compare the results with previous hypotheses. Nodal support was calculated with symmetric resampling (1000 replicates, with 10 addition sequences, saving up to 10 trees each) expressed as values of GC (groups present/contradicted), with a change probability of 0.33 (Goloboff *et al.* 2003b) and relative Bremer support (Goloboff & Farris 2001), saving up to 5000

suboptimal trees. Both support measures were calculated for implied ($K = 3$ and 6) and equal weighting.

SYSTEMATIC PALAEOONTOLOGY

Order CYPRINODONTIFORMES Berg, 1940
Suborder CYPRINODONTOIDEI Parenti, 1981
Family ANABLEPIDAE Garman, 1895

Type genus. *Anableps* Scopoli (ex Gronow), 1777.

Genus **TUCMANABLEPS** nov.

LSID. urn:lsid:zoobank.org:act:7D65E224-08E2-4D91-A042-6DA628474EF1

Type and only known species. *Tucmanableps cionei* sp. nov.

Derivation of name. Named after ‘Tucma’ and the generic name *Anableps*. Tucma was the name of a powerful cacique (tribal chief) in the Calchaquí Valley from which apparently derived the name once attributed to the whole north-western region of Argentina and today restricted to the province of Tucumán. Noun in apposition, gender feminine.

Diagnosis. The new genus is distinguished by the following combination of characters, including unique features among

Anablepidae which are marked with (*). Pronounced sexual dimorphism, males with anal-fin rays two to seven crowded together and twisted around each other forming a long gonopodium, of about 25% of standard length (*). Distal portion of gonopodium with a bilobed plate formed by an expanded portion of the sixth anal-fin ray and associated with an enlarged third anal-fin ray (*). In both males and females the dorsal-fin origin is placed three or four vertebrae posterior to anal-fin origin. In females, this point is posterior to the posterior margin of anal fin. Weakly tricuspid teeth in the premaxilla. Six branchiostegal rays. Pectoral fins located relatively high in the body, but always below the vertebral column (*). Vertebral column with 28–30 vertebrae + urophoral complex. Epipleurals associated with ribs.

Tucmanableps cionei sp. nov.
Figures 2–7

LSID. urn:lsid:zoobank.org:act:724578C8-51AC-45EC-8EB3-DF695FE4796D

Derivation of name. Species dedicated to Alberto Cione, a prominent Argentinian palaeo-ichthyologist, who was the first to study the specimens herein described.

Type material. Holotype, PVL 2412 (complete male specimen). Paratypes, complete specimens: PVL 2398 (female),



FIG. 2. *Tucmanableps cionei* gen. et sp. nov. A, holotype (PVL 2412), male specimen in right lateral view. B, paratype (PVL 3641), female specimen in right lateral view. Scale bars represent 2 mm. Colour online.

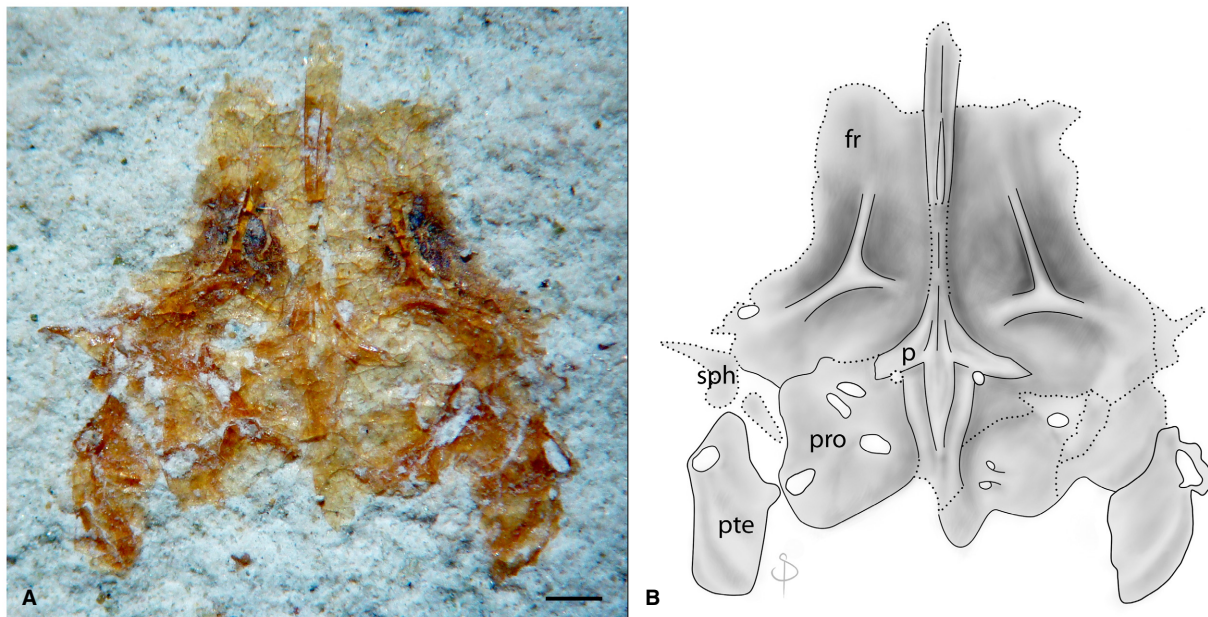


FIG. 3. *Tucmanableps cionei* gen. et sp. nov. Skull roof and braincase bones in ventral view. A, photograph of PVL 2414. B, schematic drawing of PVL 2414. Abbreviations: fr, frontal; p, parasphenoid; pro, prootic; pte, pterotic; sph, sphenotic. Scale bar represents 0.5 mm. Colour online.

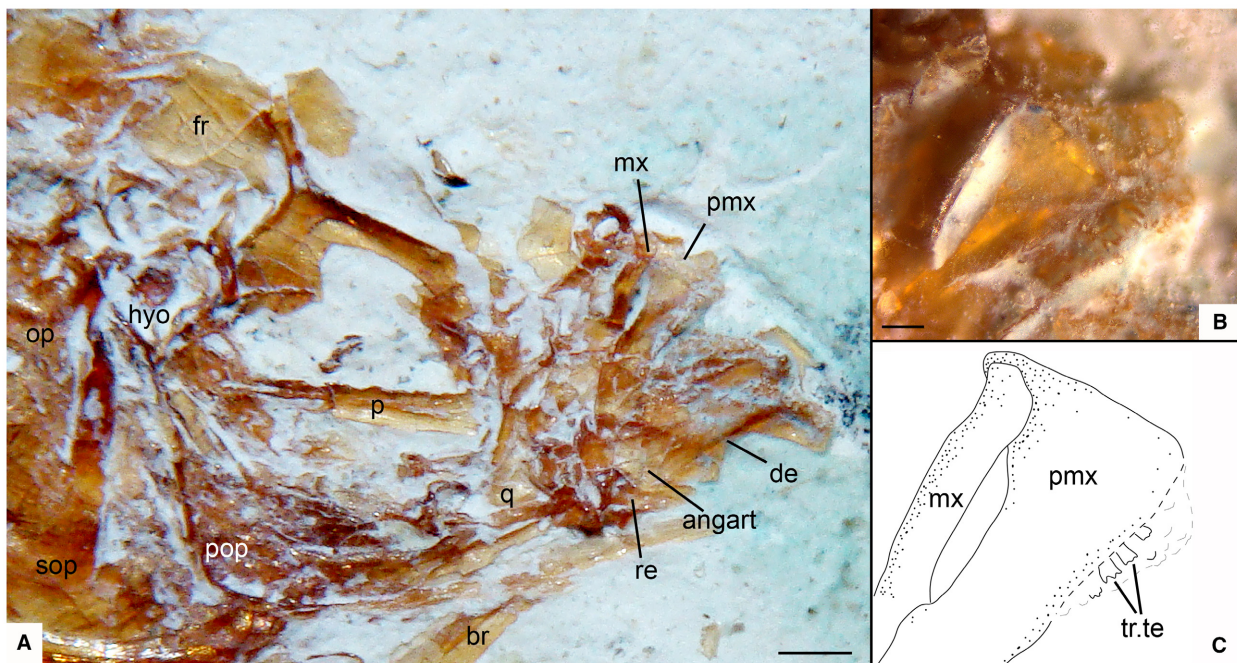


FIG. 4. *Tucmanableps cionei* gen. et sp. nov. A, anterior portion of the skull (PVL 3641). B, detailed view of premaxillary teeth (PVL 3641). C, schematic drawing of premaxillary teeth of B. Abbreviations: angart, anguloarticular; br, branchiostegal rays; de, dentary; fr, frontal; hyo, hyomandibular; mx, maxilla; op, opercle; p, parasphenoid; pmx, premaxilla; pop, preopercle; q, quadrate; re, retroarticular; sop, subopercle; tr.te, tricuspid teeth. Scale bars represent 0.5 mm (A); 0.1 mm (B). Colour online.

PVL 2410 (female), PVL 2411 (female), PVL 3579 (male), PVL 3639 (female), PVL 3641 (female). PVL 2414, isolated skull preserved in ventral view, PVL 3640 (female), almost complete specimen lacking dorsal and caudal fins.

Occurrence. Río Salí Formation of middle Miocene age (c. 12 Ma). Fish occur in levels of marls in the lower part of the Salina López member (Bossi *et al.* 1998). Specimens PVL 2398, 2410–2412 and 2414 come from the Valle de

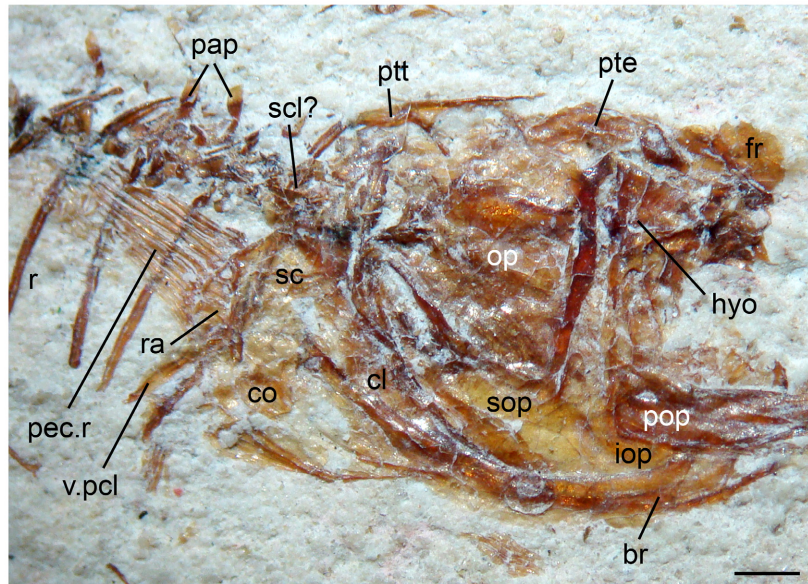


FIG. 5. *Tucmanableps cionei* gen. et sp. nov. Posterior portion of the skull, pectoral girdle and fin (PVL 3639). *Abbreviations:* br, branchiostegal rays; cl, cleithrum; co, coracoid; fr, frontal; hyo, hyomandibular; iop, interopercle; op, opercle; pap, parapophysis; pec.r, pectoral-fin rays; pop, preopercle; pte, pterotic; ptt, posttemporal; r, ribs; ra, radial; sc, scapula; scl, supracleithrum; sop, subopercle; v.pcl, ventral postcleithrum. Scale bar represents 0.5 mm. Colour online.

San Javier locality (Fig. 1, locality 2) from outcrops placed at the side of the road on the way to El Siambón, provincial route RP340, approximately 30 km from San Miguel de Tucumán, province of Tucumán (26° 39' 35.70" S, 65° 23' 33.16" W). Specimens PVL 3579, 3639–3641 were found in the Arroyo Angostura locality (Fig. 1, locality 3), west of San Pedro de Colalao of the same province (26° 14' 44.98" S, 65° 32' 14.33" W).

Diagnosis. As for genus.

Description

Tucmanableps cionei is a small-sized anablepid fish (Fig. 2). Females are larger than males, ranging from 12.4 to 25.4 mm standard length (SL), with a maximum of 18.6 mm in males and 25.4 mm in females (see Table 1). *T. cionei* has an elongated body exhibiting almost straight dorsal and ventral margins. The greatest depth is measured between the pectoral and dorsal fin origins and represents 12.7–20.5% of SL (16.2% in the holotype). Head length ranges from 23.2 to 31.6% SL (25.6% in the holotype); the lower jaw is slightly longer than the upper jaw and the mouth is terminal or slightly superior. The caudal peduncle is almost undifferentiated from the trunk. The caudal fin is rounded to truncate. Cycloid scales are present on the whole body and in a few bones of the head (opercle, preopercle, frontals).

Skull roof. Frontals are the largest bones of the skull; they are almost rectangular in shape (approximately two times longer than wide) and slightly narrower anteriorly (PVL 2398). Bones

have an even anterior margin and the postorbital region of each frontal is not expanded. The supraorbital sensory canal is clearly visible in the frontals, where it bifurcates posteriorly. Parietals were not observed in any of the specimens. Pterotics almost completely exposed in PVL 3639 (lateral view) and PVL 2414 (ventral view; Fig. 3). They are subrectangular in shape, anteroposteriorly elongated with a triangular posterior shelf, and no well-developed anterior flange. Each pterotic is perforated laterally by the temporal canal. Nasals were not observed in any specimen.

Braincase. The braincase is partially preserved in ventral view in PVL 2414 (Fig. 3). As the anterior portion is lacking, there is no information on the vomer or lateral ethmoids. The orbital portion of the parasphenoid is narrow and bears a pronounced ventral keel (PVL 3640 and Fig. 3); posteriorly it is slightly broader than in the orbital region. The ascending processes of the parasphenoid contact the prootic distally. The prootic shows a broad bridge over the trigeminofacialis chamber. Remains of a laterally narrow sphenotic are also visible (Fig. 3). Other braincase bones were not observed.

Circumorbital bones. The lacrimal and dermosphenotic are visible though badly preserved in PVL 2411 and 2412, respectively. Their exact shape was not clearly observed.

Upper and lower jaws. The premaxilla has a long, subtriangular ascending process which is perpendicular to the main axis of its alveolar arm (PVL 2411 and 3641; Fig. 4A). The alveolar arm of the premaxilla is ventrally curved and does not bear a dorsoposterior process; it has a rounded posterodorsal outline (Fig. 4A). A single tooth is visible in the holotype, which has an expanded distal portion and a central cusp and, although lateral accessory cusps

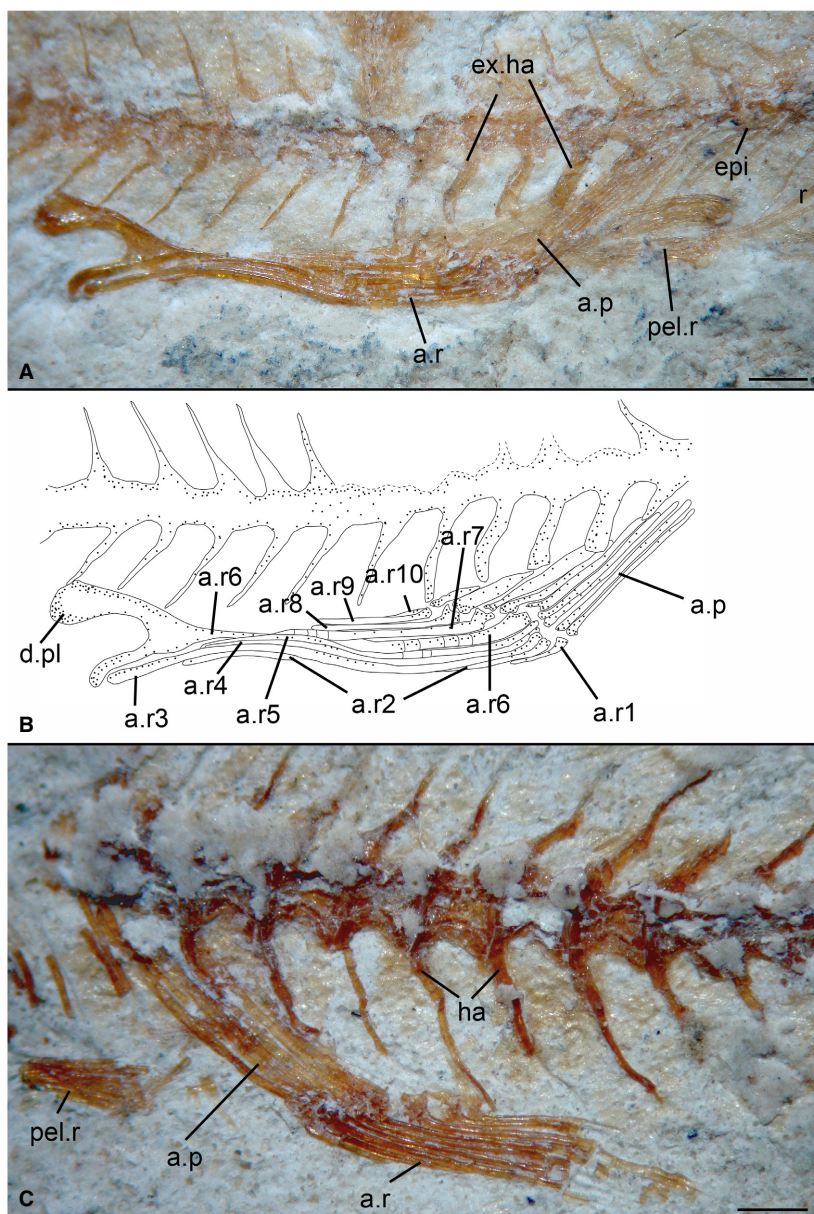


FIG. 6. *Tucmanableps cionei* gen. et sp. nov. A, detail of male gonopodium (holotype, PVL 2412). B, interpretative drawing of gonopodium of PVL 2412. C, female anal fin (PVL 3640). *Abbreviations:* a.p, anal-fin pterygiophores; a.r1–10, anal-fin rays 1–10; d.pl, distal plate; epi, epipleurals; ex.ha, expanded haemal spines; ha, haemal spines; pel.r, pelvic-fin rays; r, ribs. Scale bars represent 0.5 mm. Colour online.

were not clearly observed in this specimen, weakly tricuspid teeth are present in the premaxilla of PVL 2411 and 3641 (Fig. 4). Each tooth has a central cusp slightly larger than the accessory cusps and they are all of the same height (Fig. 4B, C). According to their size, there might have been six or seven teeth in the outer row of the premaxilla (Fig. 4B, C) and there are at least two tooth rows in the premaxilla of PVL 2411 and in the dentary and premaxilla of PVL 3641 (Fig. 4B, C). The maxilla is straight and slightly expanded distally (PVL 2411).

The dentary is rather deep, though narrower at the symphysis, and it is not expanded ventrally (Fig. 4A). It has a well developed posteroventral process. The anguloarticular has a small anterior cleft, which does not extend posteriorly beyond the posterior border of Meckel's cartilage (defined by its osseous support, PVL 3639). The anteroventral process of the

anguloarticular is not expanded but it is slightly oriented ventrally. The retroarticular is elongated and narrow (PVL 3641; Fig. 4A).

Hyopalatine bones. The quadrate has an elongated posteroventral process and a concave dorsal margin (PVL 3639–3641; Fig. 4A). It has a conspicuous articular condyle in its anterior tip. The palatine has a narrow anterior articular head and a short posterodorsal process and the dermopalatine has a straight anterior margin. The dorsal articular portion of the hyomandibula is well exposed in PVL 3639 and 3640; its anterior margin is not expanded anteriorly but approximately straight (Figs 4A, 5).

Opercular bones. The preopercle is L-shaped, with an expanded anteromedian flap. A distinct canal follows the posterior and

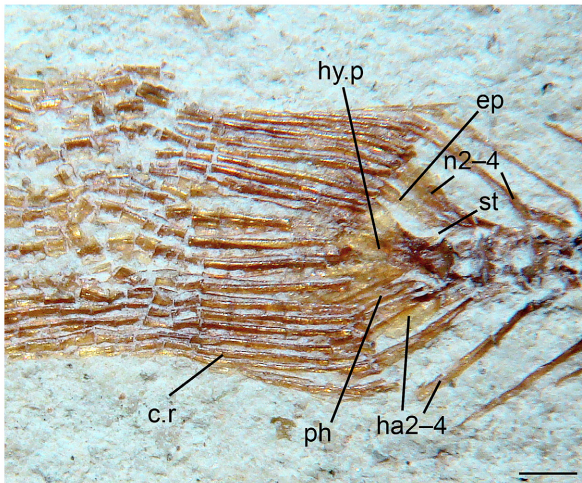


FIG. 7. *Tucmanableps cionei* gen. et sp. nov. Detail of caudal fin (PVL 3639). Abbreviations: c.r, caudal-fin rays; ep, epural; ha, haemal spines; hyp, hypural plate; n, neural spine; ph, parhypural; st, stegural. Scale bar represents 0.5 mm. Colour online.

ventral margins of the preopercle. The opercle is subtriangular, with a robust anterior border (Fig. 5) and a convex dorsal margin. The subopercle is longer than deep, around half the depth of the opercle. It bears a well-developed anterior process, which is higher than long. The interopercle has a rounded posterior margin, placed posterior to the posterior margin of the preopercle (Fig. 5). Its exact shape could not be observed.

Hyoid and branchial arches. *Tucmanableps cionei* has six branchiostegal rays (PVL 2412), four of them articulate with the anterior ceratohyal and the remaining two with the posterior ceratohyal. Other ossifications of the branchial apparatus were not clearly visible.

Vertebral column. The vertebral column is composed of 28–30 vertebrae + urophoral complex (formed by the fusion of preural and ural centra with the hypural plate). There are 10–12 abdominal vertebrae and 17–20 caudal vertebrae. Parapophyses of the abdominal vertebrae are large, longer than the length of the centrum (PVL 2398), associated with the ribs and also with thin epipleural bones (Fig. 6A). Although there are no gonapophyses related to the anal fin, the first four haemal spines are expanded in comparison to the following ones and are associated with the proximal pterygiophores of the anal fin (Fig. 6A).

Pectoral girdle and fin. *Tucmanableps cionei* has relatively highly positioned pectoral fins, though always placed below the longitudinal line defined by the vertebral column. The pectoral girdle and fin are best preserved in PVL 3639 (Fig. 5). The posttemporal bone has a long dorsal arm and a shorter and anteroventrally directed ventral arm. The supracleithrum is incompletely preserved posterodorsal to the cleithrum and ventral to the posttemporal. The cleithrum has a posterodorsally expanded lamella that does not reach caudally the vertical line

that passes through the anterior margin of the proximal radials. Although its exact shape is not clear, the cleithrum has well developed anterior and posterior flanges. The ventral postcleithrum is expanded and, thus, broader than the adjacent ribs; a small dorsal postcleithrum is present. There are four proximal radials and large scapulocoracoid bone(s) (the two bones are not clearly differentiated from each other, but probably due to their preservation) with an expanded scapular portion and a rostrally elongated coracoid portion (Fig. 5). The coracoid bears a pointed process that borders the pectoral radials ventrally. Each pectoral fin has a rounded distal border and is composed of 11 or 12 long rays which extend caudally for at least the length of four vertebrae (Fig. 5). Rays are distally segmented and branched.

Pelvic girdle and fin. Pelvic fins of *T. cionei* are positioned at 45–50% SL in both males and females in an abdominal position (Fig. 2) originating between the distal tips of the ribs associated with the seventh and eighth vertebrae. Pelvic bones (basipterygia) are subtriangular in shape, with a lateral outgrowth slightly larger than the medial outgrowth. Each pelvic bone bears a rather large medial process (PVL 3641). A posterior process is absent. Pelvic fins have six rays each that are segmented and branched only in the distal portion of the fin.

Anal fin. In both males and females the anal-fin origin is positioned at 55–62% of the SL, three to four vertebrae anterior to the dorsal-fin origin (Table 1). The male anal fin is modified to form an enlarged gonopodium (about 25% of SL) that extends caudally opposite the dorsal fin, from the haemal spine of the first to that of the eleventh caudal vertebrae (Figs 2A, 6A). This fin is composed of ten rays but only rays two to seven crowd together and twist around each other forming the gonopodium, whereas the remaining rays are shorter and straight (Fig. 6A, B). Rays three and six are longer than rays two, four, five and seven; and together they constitute the distal portion of the gonopodium. Ray six is the longest, segmented in its proximal quarter but not in its distal quarter and, remarkably, its distal portion is expanded in a bilobed plate that is unique in the order (Fig. 6B). The dorsal lobe of this plate has a rounded outline and is bigger than the ventral, which is straighter and has a slightly recurved distal tip. Both lobes are separated by a deep concavity. The plate is associated with an enlarged anal-fin ray three (Fig. 6A) and together they delimit a canal that may have been occupied by the distal portion of the spermatic duct. In the two male specimens of *T. cionei* there are nine anteriorly inclined anal-fin proximal pterygiophores. At least the first five pterygiophores are totally or partially fused together in a bunch (Fig. 6A) whereas the posterior pterygiophores are separated from each other and decrease in size caudally, though they are enlarged by the presence of anterior and posterior outgrowths. Distal and middle pterygiophores were not clearly observed in male specimens.

The female anal fin has a rounded outline and is composed of 9–11 unmodified rays which are distally segmented and branched (Fig. 6C). There is no enlarged anal-fin ray in females. As in males, proximal pterygiophores are anteriorly inclined.

Although proximal pterygiophores are slightly enlarged in females, they are thinner than in males and separated from each other. The seventh middle pterygiophore is larger than the adjacent middle pterygiophores.

Dorsal fin. The dorsal fin is placed at 65–70% of the SL in both males and females, its origin being posterior to that of the anal fin (Fig. 2; Table 1). The first dorsal proximal pterygiophore is located between the neural spines of vertebrae 16 and 17 in all specimens, except for PVL 3641 in which the first pterygiophore is placed between the neural spines of vertebrae 17 and 18. Apparently, there are six pterygiophores, each of which has anterior and posterior lamellar outgrowths. The dorsal fin is composed of seven to eight long rays that are distally segmented and branched.

Caudal fin. The caudal-fin margin is rounded. The caudal endoskeleton is dorsoventrally symmetrical and is composed of the urophoral complex, a single blade-like epural opposite to the parhypural, and four or five preural vertebrae (Fig. 7). The hypural plate is completely fused. There is a minute stegural fused dorsally to the anterior portion of the urophoral complex (PVL 3639; Fig. 7). Besides, there is a small hypurapophysis on the parhypural. The caudal-fin is formed by at least 4 dorsal procurent rays, 19 principal caudal-fin rays and 5 or 6 ventral procurent rays. All principal rays are segmented but only 15 are branched.

Squamation. The whole body is covered of thin cycloid scales, as are the opercle, preopercle and frontal bones. Scales have 7–11 radii.

Genus SACHAJENYNISIA nov.

LSID. urn:lsid:zoobank.org:act:179DDDE9-9F50-4D14-910D-D495C4704A09

Type and only known species. *Sachajenynsia pacha* sp. nov.

Derivation of name. Named after the Quechua word ‘sacha’ and the generic name *Jenynsia*. Sacha is a preposition meaning ‘almost’ and is commonly used colloquially as an adjective in north-western Argentina, meaning false or incomplete.

Diagnosis. The new genus is distinguished by the following combination of characters, including unique features among Anablepidae which are marked with (*). Tricuspid teeth in jaws. Incipient sexual dimorphism, male anal-fin rays grouped together forming a single pointed bunch (gonopodium-like structure) (*), of about 20–22% of standard length. Males with anal fin at about 58% SL, female anal fin placed at about 61–62% SL. Pectoral fins lowset. Vertebral column with 28–30 vertebrae + urophoral complex.

Sachajenynsia pacha sp. nov.

Figures 8–11

LSID. urn:lsid:zoobank.org:act:9968BF17-DDA1-491A-86D3-274312B9A0EA

Derivation of name. Named after ‘Pachamama’, which is a quechua word meaning ‘Mother Earth’, given to the goddess of the Incas representing the planet Earth, to which gifts were and are still given by the Andean inhabitants from Peru to northern Argentina. In Tucumán it is usual to refer colloquially both to the deity and the Earth as ‘la Pacha’. Because of their internal fertilization and viviparism, species of *Jenynsia* are commonly named ‘madrecitas’ in Argentina, which means ‘little mothers’.

Type material. Holotype PVL 7036, a complete male specimen (in the same shale also occur an undetermined postcranium and isolated skull and pelvic girdle remains). Paratypes: Complete specimens: PVL 7037 (female), PVL 7040 (male), PVL 7041 (female). PVL 7038 (male on top and undetermined at the bottom, almost complete specimens in the same shale as incomplete skulls and fins), PVL 7039 (female, anterior portion of the trunk disarticulated, isolated urophoral complex), PVL 7042 (undetermined, part of the skull and anterior portion of the trunk), PVL 7043 (undetermined, skull and anterior portion of the trunk), PVL 7044 (male, part of the skull and postcranium).

Occurrence. Río Salí Formation of middle Miocene age (c. 12 Ma). All specimens were collected in the locality Toma de Vipos (26° 29' 56" S, 65° 23' 55" W; Fig. 1, locality 1) in the province of Tucumán. Fish occur in the upper levels of marls just below the horizon rich in nodules of gypsum at the top of the outcrop (see Geological Setting).

Diagnosis. As for genus.

Description

Sachajenynsia pacha is a medium-sized anablepid fish (Fig. 8A, B). Females are slightly larger than males, ranging from 37.6 to 41.0 mm SL, whereas male SL ranges from 32.8 to 37.1 mm (Table 2). *S. pacha* has an elongated body exhibiting almost straight dorsal and ventral margins. The greatest depth is located at the origin of the pectoral fin and represents 16.4–20.2% SL. Head length ranges from 24.6% to 27.4% SL, the lower jaw is slightly longer than the upper jaw and the mouth is slightly superior. The caudal peduncle is almost undifferentiated from the trunk. The caudal fin is rounded to truncate. Cycloid scales are present on the whole body and in a few bones of the head (opercle, preopercle, frontals).

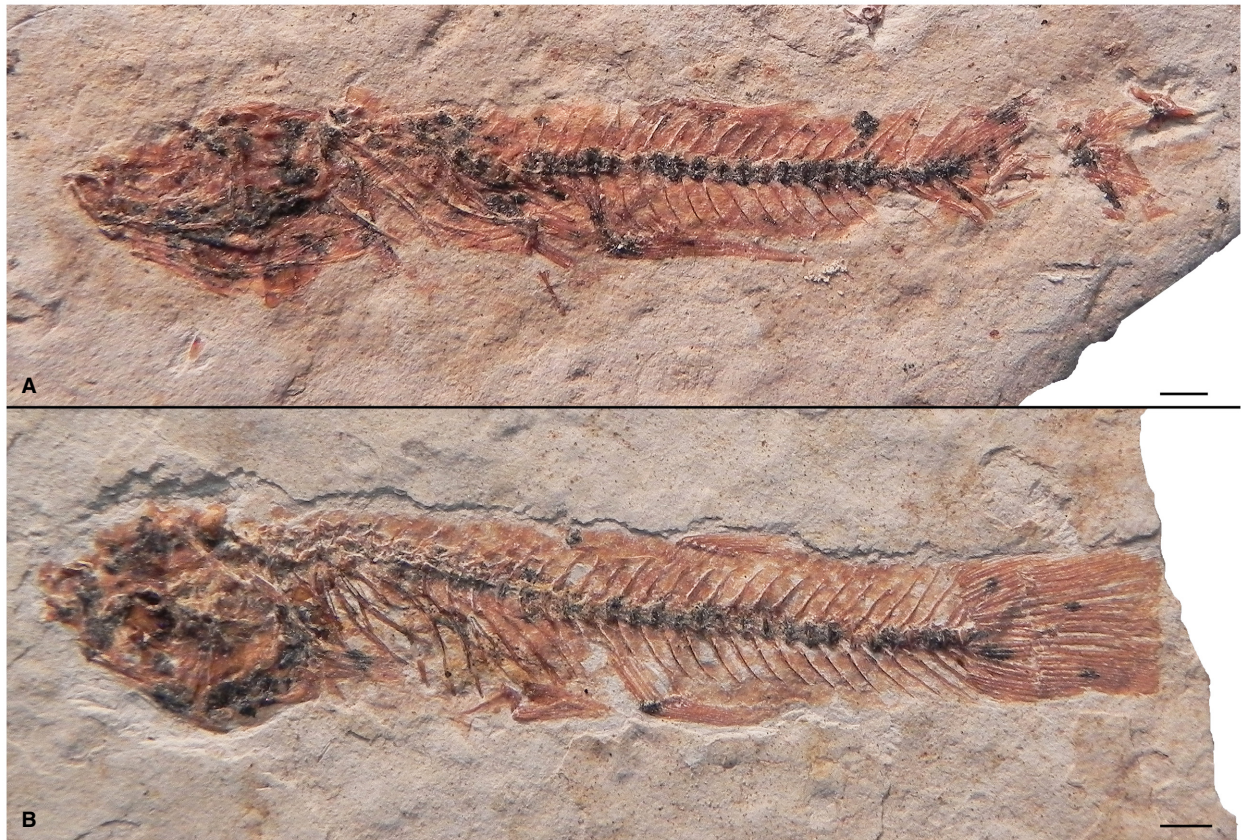


FIG. 8. *Sachajenynsia pacha* gen. et sp. nov. A, holotype (PVL 7036), male specimen in left lateral view. B, paratype (PVL 7037), female specimen in left lateral view. Scale bars represent 2 mm. Colour online.

Skull roof. Frontals are subrectangular in shape (around 2.5 times longer than deep), they are not expanded in their posterodorsal portion and its anterior margin is even (PVL 7039 and 7041; Fig. 9A). The supraorbital sensory canal bifurcates posteriorly in the frontals forming a wide Y shape. A pterotic is badly preserved in dorsal view in PVL 7039 (Fig. 9A). Its exact shape cannot be established but the pterotic exhibits a triangular posterior shelf and is perforated by the temporal canal close to its lateral margin. Parietals and nasals were not undoubtedly observed in any of the specimens.

Braincase. An articulated parasphenoid and prootic are preserved in ventral view as an isolated remnant in PVL 7036 (Fig. 9B). The parasphenoid is narrow in the orbit region, with a pronounced ventral keel. The ascending processes of the parasphenoid contact the prootic distally. The prootic forms a broad bridge over the trigeminofacialis chamber. The sphenotic is laterally narrow and does not bear a posteriorly directed process (PVL 7039 and 7041; Fig. 9A). Remains of the epioccipital, supraoccipital and part of the supraoccipital process are visible in PVL 7039 (Fig. 9A). Other braincase bones were not observed.

Circumorbital bones. The lacrimal and dermosphenotic are not preserved in any of the specimens.

Upper and lower jaws. Each premaxilla has a long and approximately triangular ascending process (PVL 7040; Fig. 10A). The exact shape of the alveolar arm of the premaxilla as well as the presence of a dorsoposterior process on it, could not be determined. The maxilla is straight and has a moderately expanded distal arm (Fig. 10A). The dentary is narrow at the symphysis and does not expand ventrally (Fig. 10A). It seems to have a rather short posteroventral process (PVL 7036). There are no preserved teeth in the holotype, but tricuspid teeth are present arranged in at least two rows in the dentary of PVL 7040 (Fig. 10A–C). The three cusps are well differentiated from each other; the central cusp is larger and much higher than the accessory ones (Fig. 10B, C). The anguloarticular has a small anterior cleft, which does not extend posteriorly beyond the posterior border of Meckel's cartilage (PVL 7037). The anteroventral process of the anguloarticular is expanded (PVL 7041). The retroarticular was not clearly observed in any specimen.

Hyopalatine bones. The palatine bone is well exposed in PVL 7040 (Fig. 10A). It has a narrow anterior articular head and bears a short posterodorsal process. The quadrate has an elongated posteroventral process and a slightly convex dorsal margin (Fig. 10A). It has a conspicuous articular head which is anterior to the rest of the quadrate body. The main shaft of the

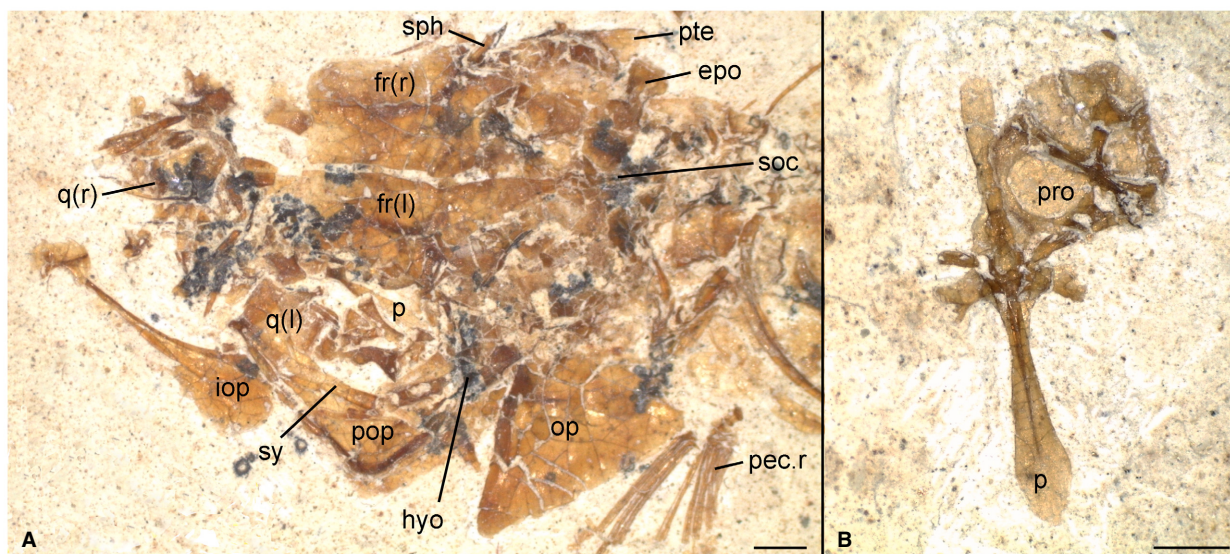


FIG. 9. *Sachajenymsia pacha* gen. et sp. nov. A, skull of PVL 7039. B, detail of braincase bones (PVL 7036). *Abbreviations:* epo, epio-cipital; fr, frontal; hyo, hyomandibular; iop, interopercle; (l), left element; op, opercle; p, parasphenoid; pec.r, pectoral-fin rays; pop, preopercle; pro, prootic; pte, pterotic; q, quadrate; (r), right element; soc, supraoccipital; sph, sphenotic; sy, symplectic. Scale bars represent 1 mm. Colour online.

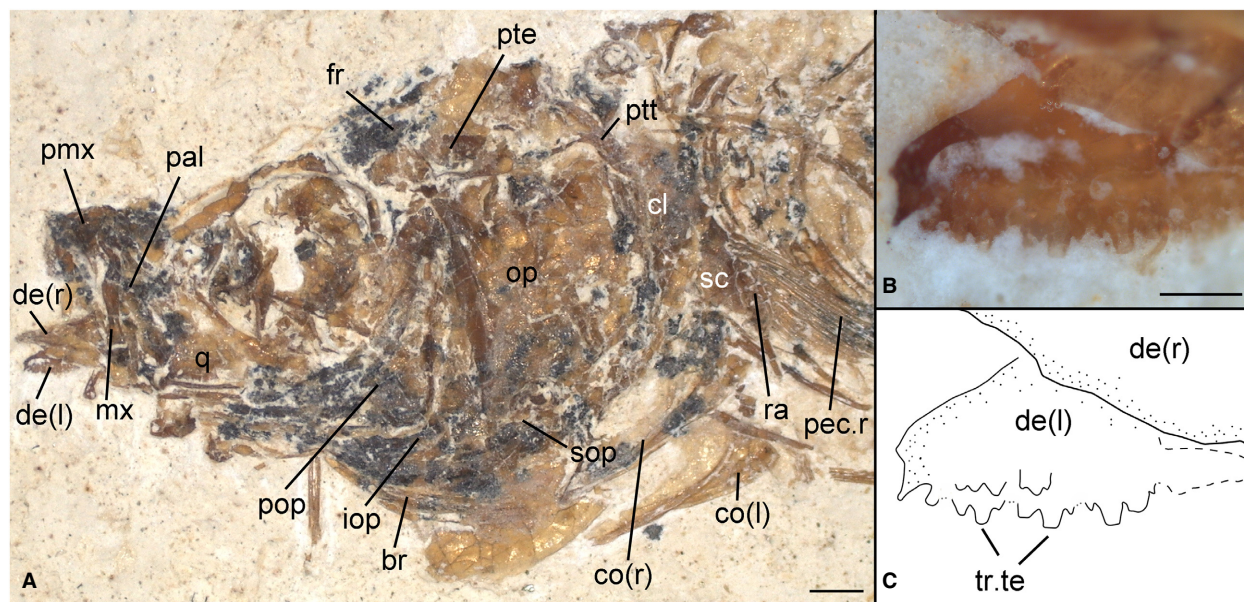
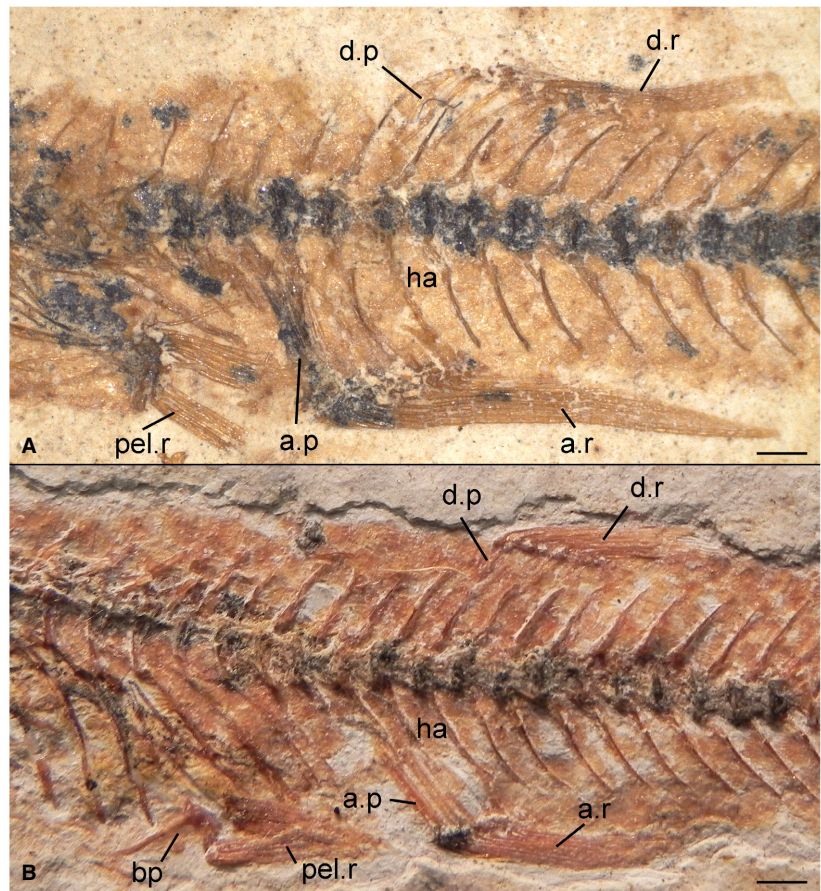


FIG. 10. *Sachajenymsia pacha* gen. et sp. nov. A, skull and anterior portion of the trunk (PVL 7040). B, detail of dentary teeth (PVL 7040). C, schematic drawing of dentary teeth of B. *Abbreviations:* br, branchiostegal rays; cl, cleithrum; co, coracoid; de, dentary; fr, frontal; iop, interopercle; (l), left element; mx, maxilla; op, opercle; pal, palatine; pec.r, pectoral-fin rays; pmx, premaxilla; pop, preopercle; pte, pterotic; ptt, posttemporal; q, quadrate; (r), right element; ra, radial; sc, scapula; sop, subopercle; tr.te, tricuspid teeth. Scale bars represent 1 mm (A); 0.2 mm (B). Colour online.

hyomandibula is anteroventrally directed. The anterior margin of this bone is not expanded but approximately straight or slightly concave (PVL 7037). The symplectic is splint-like, articulates dorsally with the hyomandibula and reaches anteriorly the quadrate (PVL 7037).

Opercular bones. The preopercle is L-shaped with an expanded and triangular anteromedian flap (Figs 9A, 10A). The preopercular canal is located nearly at the posterior and ventral margins of the preopercle and its dorsal and anterior ends are almost reduced to a canal. The opercle is a subtriangular bone, deeper

FIG. 11. *Sachajenynsia pacha* gen. et sp. nov. A, detail of male gonopodium (holotype, PVL 7036). B, female anal fin (PVL 7037). *Abbreviations:* a.p, anal-fin pterygiophores; a.r, anal-fin rays; bp, basipterygium; d.p, dorsal-fin pterygiophores; d.r, dorsal-fin rays; ha, haemal spines; pel.r, pelvic-fin rays. Scale bars represent 1 mm. Colour online.



than long. It has a robust anterior border, a well-defined acute anteroventral angle and a convexly rounded dorsal margin (PVL 7039; Fig. 9A). The subopercle is longer than deep, less than half the depth of the opercle, and bears a well-developed anterior process, which is higher than long (PVL 7038). The interopercle is a triangular, rostrally elongated bone with a rounded posterior margin (Fig. 9A). Anteriorly, it tapers reaching medially the anterior end of the preopercle.

Hyoid and branchial arches. At least four rays are present in PVL 7036. An hourglass-shaped anterior ceratohyal, part of a large ventral hypohyal and part of the posterior ceratohyal are exposed in PVL 7037. Other ossifications of the branchial apparatus were not clearly visible.

Vertebral column. The vertebral column is composed of 28–31 vertebrae + urophoral complex. There are 11–12 abdominal vertebrae and 18–20 caudal vertebrae. The parapophyses of the abdominal vertebrae are moderate in length, not longer than the length of the centrum (PVL 7039). There are no intermuscular bones associated with the column.

Pectoral girdle and fin. Pectoral fins are set low on the body. The pectoral girdle and fin are best preserved in PVL 7036 and 7040 (Fig. 10A). The posttemporal bone has a dorsoventrally

narrow main body, a long dorsal arm and a shorter and anteroventrally directed ventral arm. The supracleithrum is badly preserved in all specimens. The cleithrum has a posterodorsally expanded lamina that reaches caudally the vertical line that passes through the anterior margin of the proximal radials (Fig. 10A). The cleithrum has well developed anterior and posterior flanges (isolated bone in PVL 7036). The number and exact shape of the postcleithra were not clearly observed. Coracoids are well exposed in PVL 7040 (Fig. 10A). Each coracoid is a triangular, rostrally elongated bone, with a robust ventral margin and a gently concave dorsal margin. Posterodorsally, it articulates with the rectangular scapula. The four proximal radials are mostly associated with the scapula although the ventralmost one (fourth radial) is close to the coracoid (Fig. 10A).

Pectoral fin with a rounded distal border and composed of 12–14 segmented distally branched rays (PVL 7036 and 7040; Fig. 10A).

Pelvic girdle and fin. Pelvic fins of *S. pacha* are placed at 50% of the SL in both males and females in an abdominal position (Fig. 8A, B). Each fin is inserted between the distal tips of the ribs associated to the seventh and eighth vertebrae. Pelvic bones are subtriangular in shape, with approximately equal lateral and medial outgrowths. Pelvic bones bear a rather large medial process (PVL 7041 and 7042) but the presence of a posterior

process was not confirmed. Each pelvic fin has six branched rays (PVL 7041).

Anal fin. The anal-fin origin is slightly posteriorly in females (61–62% SL) as compared to males (58% SL; Table 2), at two to three vertebrae anterior to dorsal-fin origin (Fig. 11A, B). In both males and females, the anal fin has 10–11 rays, of which the first and last are shorter than the others. Although the male anal fin is not obviously modified into a gonopodium (as known in all extant anablepid species with the exception of *Oxyzygonectes dovii*), there is an evident sexual dimorphism between female and male anal fins as in males all rays are tightly grouped together forming a single pointed bunch (PVL 7036, 7038 and 7040; Fig. 11A). Except for the first ones, which are unbranched, all rays are segmented and distally branched. This gonopodium-like array of anal-fin rays extends caudally from 20% to 22% SL (from the haemal spine of the first to that of the tenth caudal vertebra) ending in a distal tip. There are nine proximal pterygiophores (PVL 7038 and 7040; Fig. 11A), which are parallel to one another. The first four pterygiophores are slightly enlarged and fused together in a bunch (Fig. 11A) whereas the posterior pterygiophores are separated from each other and decrease in size caudally.

In the female anal fin, the rays are not grouped in a bunch (Fig. 11B). First rays are unbranched whereas the consecutive ones are branched. There are 9–10 proximal pterygiophores; the first ones are slightly enlarged, but thinner than male anal-fin pterygiophores and they are separated from each other.

Dorsal fin. The dorsal-fin origin is at 62–65% of the SL. In males the dorsal-fin origin is opposite the posterior section of the anal-fin base; whereas in females its origin is opposite the centre of the anal fin base (Fig. 11A, B). The first dorsal proximal pterygiophore is located between the neural spines of vertebrae 14–15 in male specimens and between the neural spines of vertebrae 15–16 in females. There are seven or eight proximal pterygiophores, each of which has a minute anterior and posterior lamellar outgrowth (PVL 7041). The first pterygiophore is triangular in shape. Dorsal fin with eight branched rays.

Caudal fin. The caudal-fin margin is slightly rounded. The urophoral complex is formed by a single hypural plate fused with first preural centrum and ural centra, and a single blade-like epural opposite to the parhypural, and four to five preural vertebrae. The hypural plate is completely fused. There is a minute stegural fused dorsally to the anterior portion of the urophoral complex (isolated urophoral complex in PVL 7039). Besides this, there is a small hypurapophysis on the parhypural (PVL 7039). The caudal fin is formed by at least 8 dorsal procurent rays, 19–20 principal caudal-fin rays and 6–7 ventral procurent rays. All principal rays are segmented but about 16 or 17 are branched.

Squamation. The whole body is covered of thin cycloid scales as are the opercle, preopercle and frontal bones. Scales also cover a few bones of the head (opercle, preopercle, frontals) but their preservation does not allow the identification of head-squamation type, as in living representatives of the family. Scales have around 7–11 radii (PVL 7036).

PHYLOGENETIC RESULTS

Under equal weights, 12 equally most parsimonious trees of 163 steps were found (CI = 0.54; RI = 0.76). *Sachajenynsia pacha* is recovered as the sister taxon of a clade composed of *Tucmanableps cionei* and the genera *Anableps* and *Jenynsia* (Fig. 12A). *Tucmanableps cionei* is the sister taxon of *Anableps* (a clade composed of *A. dowi* as sister species of *A. anableps* + *A. microlepis*). The topology of the consensus tree is the same as that proposed by Aguilera et al. (2013) for the genus *Jenynsia*.

Under implied weighting, a single most parsimonious tree of 165 steps (Fit: 14.64643; CI = 0.54; RI = 0.75) was obtained with a concavity constant of 3 (K3) and two equally parsimonious trees of 163 steps (Fit: 9.12359; CI = 0.546; RI = 0.758) with K6. The strict consensus of the two equally parsimonious trees calculated with K6, as well as the single most parsimonious one obtained with K3, is provided as Figure 12B, C.

Clades supporting the sister-group relationship of *T. cionei* with *Anableps* and *S. pacha* with all remaining internal fertilizing genera of anablepids are relatively well supported and are herein considered to be reliable monophyletic groups.

DISCUSSION

Systematic position of Tucmanableps cionei and Sachajenynsia pacha

The positioning of the new taxa in the Order Cyprinodontiformes is supported by the presence in both new species of a rounded caudal-fin margin, more than eight branched principal caudal-fin rays, principal caudal-fin rays continuous on upper and lower hypural plates, a symmetric caudal endoskeleton formed by the urophoral complex (hypurals + first preural centrum and ural centra) as well as a single blade-like epural opposite to the parhypural, totally or partially fused hypural plates, four to five preural vertebrae forming part of the caudal endoskeleton, fully developed neural spine on second preural centra, minute stegural and three to seven branchiostegal rays (Rosen & Bailey 1963; Parenti 1981; Costa 1998). As in other cyprinodontoids, females of *T. cionei* and *S. pacha* are generally larger than males, there is a small hypurapophysis in the caudal fin and they have cycloid scales with up to 16 radii (Parenti 1981; Costa 1998).

Apart from the presence of a male anal fin modified into an intromittent organ or gonopodium in *T. cionei* and *S. pacha*, which is characteristic of Poeciliinae and Anablepinae and is discussed below, the new taxa show many anablepid diagnostic features. The Anablepidae are

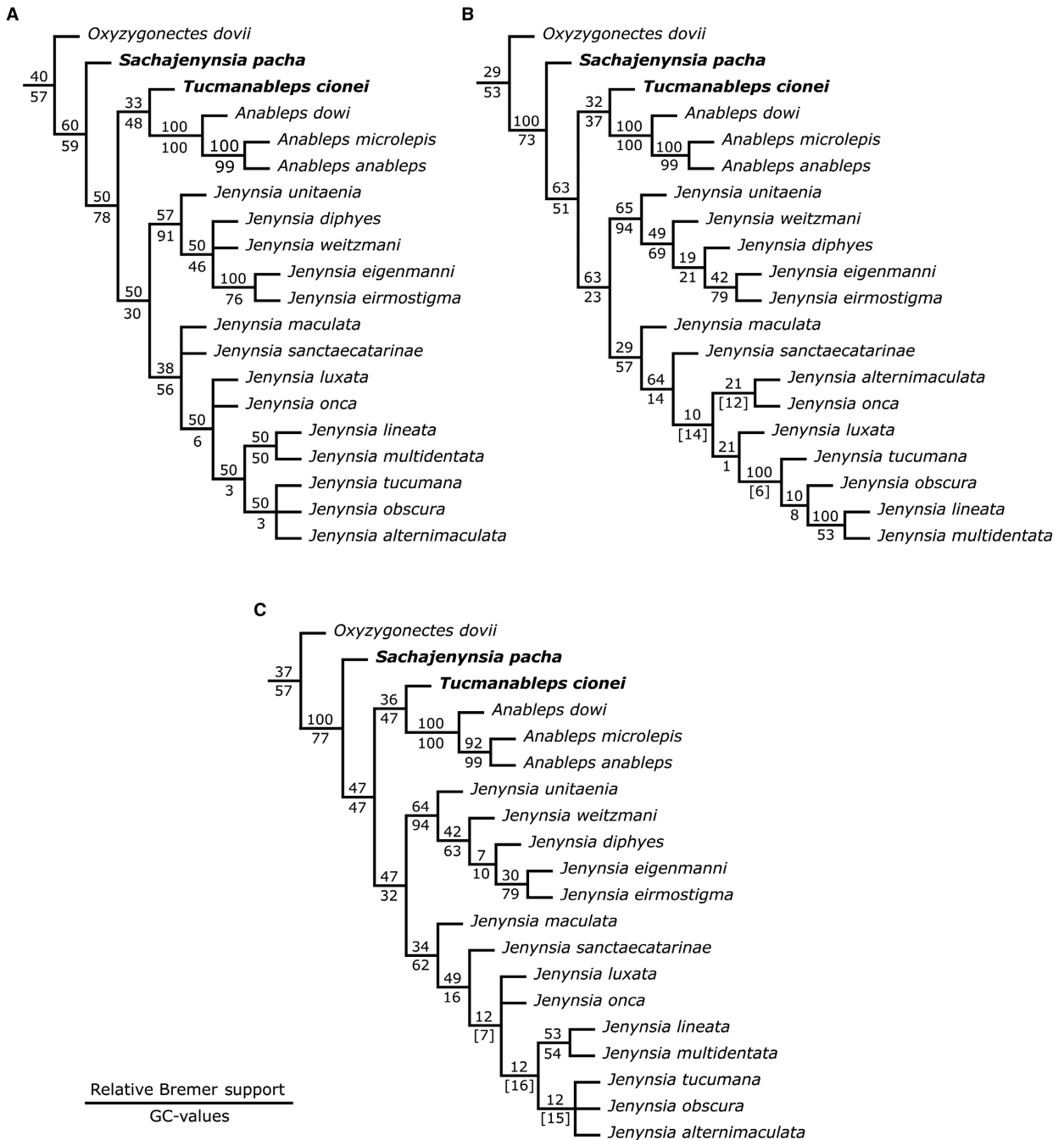


FIG. 12. Topologies from most equally parsimonious trees and relative Bremer support and GC values. A, calculated under equal weights (strict consensus of 12 trees). B, calculated under implied weighting using a concavity $K = 3$ (single most parsimonious tree). C, calculated under implied weighting using a concavity $K = 6$ (strict consensus of two trees).

uniquely diagnosed within the Poecilioidea and differ from other cyprinodontiform fishes by the possession of tricuspid teeth in embryos and young individuals (absent in some *Anableps*, see below), a prominent posteroventral process of the hyomandibula, a broad ventral postcleithrum, sexual laterality in males (Ghedotti 1998), robust

epioccipital and supraoccipital processes and pelvic fins in abdominal position (*contra* anteriorly placed pelvic fins in poeciliids; Parenti 1981). Many of these features could not be observed in the new taxa here described, like the occurrence of a prominent posteroventral process of the hyomandibula as well as the condition of epioccipital and

supraoccipital processes which remain unknown. *Tucmanableps cionei* shows a broad ventral postcleithrum and sexual laterality in males (the distal plate of the gonopodium is slightly curved laterally), conditions unknown for *S. pacha*, however, both *T. cionei* and *S. pacha* have abdominal pelvic fins and tricuspid teeth in the jaws. In particular, the premaxillary teeth of *T. cionei* are weakly tricuspid, as described for the outer row of teeth of *Oxyzygonectes* and that of the juvenile of *Anableps dowi* Gill, 1861 by Parenti (1981). The other two *Anableps* species have unicuspid teeth in outer and inner rows of the jaws (Ghedotti 1998). In contrast, the teeth of *Sachajenysia pacha* have three well differentiated cusps where the central cusp is remarkably larger and higher than the accessory ones, as figured for *Jenynsia* species (see e.g. Costa 2011, fig. 3A). The presence of at least two rows of tricuspid teeth in the premaxilla and dentary of the new taxa also resembles the condition observed in anablepids, where the jaws have an external row of larger teeth and several rows of smaller teeth irregularly arranged (*contra* tricuspid dentition represented in a single row in most cyprinodontids; Costa 2011).

In the proposed phylogenetic hypothesis, *Oxyzygonectes dovii* appears basal to the two taxa herein described. *S. pacha* and *T. cionei* share some features not present in *O. dovii*, such as an even anterior frontal margin (*contra* extending anteriorly between nasals), a short posterodorsal process of palatine (*contra* a long process), 10–11 anal-fin rays (*contra* 13–16) and the hypural plate fused as a single element (*contra* fused, forming separate symmetrical dorsal and ventral hypural elements).

Even though *S. pacha* was recovered as the sister taxon of the Anablepinae (*T. cionei*, *Anableps* and *Jenynsia*) in our phylogenetic analysis, the overall morphology of *S. pacha* is more similar to that of *Jenynsia* species than to *Anableps*. *S. pacha* shares with the clade formed by *T. cionei*, *Anableps* and *Jenynsia* the number of anal-fin rays, an unbranched anal-fin ray three in females (branched in *T. cionei*) and an anteriormost proximal anal-fin pterygiophore fused to the subsequent proximal pterygiophores forming a structure composed of five or six fused pterygiophores in males.

Tucmanableps cionei shares with *Anableps* species the occurrence of a rounded posterodorsal process on the alveolar arm of the premaxilla; enlarged paraphophyses of abdominal vertebrae, longer than the length of the centrum; the absence of a posterior process on the pelvic bone and the dorsal-fin origin positioned at a vertical that passes posterior to the anal-fin base, whereas in all *Jenynsia* species the origin of the dorsal fin is slightly anterior to or occurs over the centre of the anal fin (Ghedotti 1998). Besides these characters that support the grouping of *T. cionei* with *Anableps*, these two taxa share the possession of six branchiostegal rays. However, *T. cionei*

differs from *Anableps* species and shares with *Jenynsia* species the low number of vertebrae (28–30 in the new taxon and most *Jenynsia* species *contra* 45–54 in *Anableps*), a lower number of pectoral-fin rays (less than 19 *contra* 19 or more in *Anableps*) and in the general shape of the frontal bones, which lack an expanded postorbital region to allocate the eyes as in *Anableps*. This morphology suggests that the eyes of *T. cionei* were not located as dorsally as those of *Anableps* and most probably also lacked the significant eye modifications that allow simultaneous vision within and outside the water.

Oldest record of internal fertilization in Anablepidae

Viviparism in the Cyprinodontidae was optimized as appearing in parallel in the clade of *Anableps* and *Jenynsia* (Anablepinae) and the Poeciliinae (Poeciliidae) both in specific phylogenetic hypotheses (e.g. Ghedotti 2000) and in large-scale ones (e.g. Miranda 2017). The anal fin of mature males in the viviparous species of both families is modified as an intromittent organ (gonopodium), but morphological details of this organ differs in the two families, which is congruent with the hypothesis of two parallel acquisitions of this reproductive mode. The intromittent organ of the Anablepinae (formed by all anal-fin rays crowding together and twisting around each other in *Anableps* and by anal-fin rays three and five to seven in *Jenynsia*) involves more anal-fin rays than in the Poeciliinae, but the gonopodium itself and the associated structures are comparatively simpler. In particular, the extant Anablepinae lack a distal plate or soft-tissue structure associated with the gonopodium, as observed in some poeciliids (e.g. in *Tomeurus* Eigenmann, Ghedotti 2000, fig. 15B or *Phalloceros* Eigenmann, Lucinda 2008, fig. 9) and do not bear a gonactinostean complex (Rosen & Bailey 1963, fig. 14).

Even though the gonopodium morphology present in *Tucmanableps cionei* and *Sachajenysia pacha* is distinct from that observed in living anablepines or poeciliines, it is clearly different to that of poeciliines as in both fossil species the gonopodium is formed by more than three fin rays, like in anablepines. Besides, there is no gonactinostean complex related to the gonopodium of the new fossil taxa, but only slightly enlarged haemal arches associated with the intromittent organ.

The incipient gonopodium of *S. pacha* is very simple and thus cannot be undoubtedly related to that of *Anableps* or *Jenynsia*. Interestingly, Ghedotti (1998) proposed a hypothesis concerning the evolution of the Anablepinae (*Anableps* + *Jenynsia*) gonopodium based on his phylogeny (Ghedotti 1998, fig. 25) and unambiguous character-state transformations. That author hypothesized that a tubular gonopodium was present in the ancestor of the

Anablepinae, which had no reduced anal-fin rays and was supported by anteriorly inclined proximal pterygiophores. This description agrees with our observations of the anal fin of *S. pacha*, which is here recovered as the sister group of the internal fertilizing genera (Anablepinae) in our phylogenetic analysis. Although it is impossible to assess whether the anal fin of *S. pacha* served as a true gonopodium, it shows a sexual dimorphism that could be related to sperm transfer.

On the other hand, the gonopodium of *T. cionei* is formed by rays 2–7 that crowd together and twist around each other, resembling the condition observed in *Anableps*, at least in the number of rays involved (Ghedotti 1998, fig. 17A). However, *T. cionei* exhibits a bilobed plate in the distal end of the gonopodium, which is absent in extant anablepines. Somewhat similar structures are present in the distal end of the gonopodium of some poeciliines, but those gonopodial appendices are paired (Rosen & Bailey 1963; Ghedotti 2000; Lucinda 2008) and herein considered not homologous to the bilobed plate of *T. cionei*. Contrary to *S. pacha*, in *T. cionei* internal fertilization is more plausible, with males bearing a gonopodium that could be more complex than in any other known anablepid. Though we have no evidence of viviparity in the taxa herein described, all the extant anablepids having internal fertilization are viviparous and, when optimizing this feature in the obtained phylogenetic trees, it is most parsimonious to think that *T. cionei* was viviparous. However, viviparity is ambiguously optimized for the clade including *S. pacha* with the remaining Anablepinae and this condition cannot be assessed or inferred for this species with the available information.

The new taxa herein described show that the fossil diversity of the Anablepidae is probably larger than the extant and previously known fossil one. In this context, the Río Salí Formation has recorded an important stage of diversification of the Anablepidae, when distinct and unique morphologies had arisen and the group was already demonstrating sexual reproduction by means of internal fertilization. In the middle Miocene, the open lake inhabited by *T. cionei* and *S. pacha* was associated with the Entrerriean–Paranense Marine Ingression (Aceñolaza & Sprechmann 2002) which is congruent with the idea that the distribution and diversification of cyprinodontiform fishes are usually related to marine or brackish waters (Aguilera & Mirande 2005; Calviño & Alonso 2016).

CONCLUSION

The Anablepidae are currently a small family, with only three genera that are highly morphologically divergent from one another. The two genera described herein are

unique among the Anablepidae in showing an incipiently developed gonopodium (*S. pacha*) and bearing the most complex known intromittent organ in the family (*T. cionei*). In extant genera of the family, the modification of the anal fin into an intromittent organ is related to internal fertilization. Even though internal fertilization cannot be undoubtedly confirmed for *S. pacha*, it was clearly the reproductive mode of *T. cionei*.

Sachajenyasia pacha is recovered as the sister group of all anablepids with evident internal fertilization, whereas *T. cionei* forms a clade with *Anableps* species. The existence of two new anablepid genera showing distinct and unique morphologies suggests that the fossil diversity of the Anablepidae may be higher than the extant one. Finally, its occurrence in open lake deposits with coastal facies and inner lake facies associated with the Miocene Entrerriean–Paranense Marine Ingression is congruent with the habitat type of extant members of the family and the current ideas about the diversification of the Anablepidae.

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DATA ARCHIVING STATEMENT

The data matrix for the present study is available in MorphoBank: <https://morphobank.org/permalink/?P2726>. This published work and the nomenclatural acts it contains, have been registered in ZooBank: <http://zoobank.org/References/EA4F7704-AB96-4FE9-A6B2-9D304D37F2B2>

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