

## A new deep-sea eelpout of the genus *Pyrolycus* (Teleostei: Zoarcidae) associated with a hydrothermal seep on the Pacific margin of Costa Rica

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### Abstract

A new species of the zoarcid genus *Pyrolycus* Machida & Hashimoto, 2002, *Pyrolycus jaco* **sp. nov.**, is described from a hydrothermal seep environment named Jacó Scar in the eastern Pacific of Costa Rica. Four specimens were collected in 2018 between 1746–1795 m among tubeworm colonies around the seep. The new species is differentiated from its two western Pacific congeners by having a shorter head, snout, jaw, and pectoral fins. It is further diagnosed by having three postorbital pores and two occipital pores. Molecular sequences of the cytochrome c oxidase I gene are provided and are the first for the genus. The character states indicating miniaturization in this species are discussed. This is the first vertebrate species known from this composite reducing ecosystem and is the fourth hydrothermally-associated zoarcid from the eastern Pacific.

**Key words:** Jacó Scar, Lycodinae, methane seep, Reducing ecosystem, Zoarcoidei

### Introduction

Zoarcidae is one of seven fish families with members that have adapted to live in association with deep-sea reducing ecosystems around hydrothermal vents and methane seeps (Priede 2017). Three zoarcid genera, all in the subfamily Lycodinae Gill, 1861, are currently known to be endemic or near-endemic to vent and seep environments (Priede 2017; Thiel *et al.* 2021): *Pachycara* Zugmeyer, 1911 (seven endemic species, one near-endemic, known from vents and seeps), *Pyrolycus* Machida & Hashimoto, 2002 (two species, known from vents), and *Thermarces* Rosenblatt & Cohen, 1986 (three species, known from vents).

During five submersible expeditions conducted between 2009–2019 to methane seeps on the Pacific margin of Costa Rica, an unknown zoarcid species was observed (Levin *et al.* 2012) and collected at a unique seep known as Jacó Scar. Geologically characterized as a seamount subduction scarp (Bohrmann *et al.* 2002) with high methane concentrations (Mau *et al.* 2012), the Jacó Scar seep is considered a composite reducing habitat in that it also exhibits vent-like characteristics, namely elevated temperatures up to 5.2 °C (approximately 3 °C above ambient) and the presence of vent-affiliated fauna (Levin *et al.* 2012). For example, the mussel *Bathymodiolus thermophilus* Kenk and Wilson, 1985, was previously known only from hydrothermal vents until new records were confirmed from the Jacó Scar “hydrothermal seep” (Levin *et al.* 2012; McCowin *et al.* 2020).

The Jacó Scar fish were observed at densities up to 28 ind. m<sup>-2</sup> living among colonies of the tubeworms *Lamellibrachia barhami* Webb, 1969 and *Escarpia spicata* Jones, 1985 (Levin *et al.*, 2012). The fish were not observed beyond the colonies around the seep or on nearby non-thermal seeps (G. Rouse, pers. comm.). Using a

sequence of the mitochondrial cytochrome c oxidase I gene (COI), Levin *et al.* (2012) tentatively assigned these fish to the genus *Pachycara* based on a BLAST match of 94% identity to *P. thermophilum* Geistdoerfer, 1994 but did not investigate the taxonomy of the species further. In 2018, additional specimens were collected by the HOV *Alvin* between 1746–1795 m. The specimens possess an L-shaped pattern of their suborbital bones and a weak oral valve and lack an interorbital pore placing them in Lycodinae, which represents the bulk of zoarcid diversity with 33 genera and 245 species (Fricke *et al.* 2022).

The Jacó Scar specimens lack scales and a lateral line as in *Pyrolycus* and *Thermarces* while these characters are present in most species of *Pachycara* (Anderson 1994; 2006). They possess gelatinous flesh and pelvic fins (lacking in *Thermarces*) and have a relatively long preanal length (vs. shorter in *Pachycara*) and a low vertebral count (~80 vs. 92+ in *Pachycara* and *Thermarces*). These characters place them in the genus *Pyrolycus*, previously known from only two species from hydrothermal vents in the western Pacific, *P. manusanus* Machida & Hashimoto, 2002 from the Bismarck Sea and *P. moelleri* Anderson, 2006 from the Kermadec Ridge. The specimens do not correspond to either species and are herein described as new. They differ from both congeners by having a shorter head, snout, jaw, and pectoral fins. Additionally, the specimens have two occipital pores vs. one or absent in the other two species. Molecular sequences of COI are provided and are the first for the genus. The new species of *Pyrolycus* is the first vertebrate species known from this composite reducing ecosystem and is the fourth hydrothermally-associated zoarcid from the eastern Pacific, the others being *Pachycara rimae* Anderson, 1989, *Thermarces andersoni* Rosenblatt & Cohen, 1986, and *T. cerberus* Rosenblatt & Cohen, 1986.

## Material and methods

Specimens were observed during interdisciplinary research cruises to the Pacific margin of Costa Rica: R/V *Atlantis* with DSV *Alvin* AT15-44 (2009), AT37-13 (2017), AT42-03 (2018); R/V *Falkor* with ROV *SuBastian* FK190106 (2019). Specimen collection and field operations were performed under the following permits issued by CONAGEBIO (Comisión Nacional para la Gestión de la Biodiversidad), INCOPECA (Instituto Costarricense de Pesca y Acuicultura), and SINAC (Sistema Nacional de Áreas de Conservación) under MINAE (Ministerio de Ambiente y Energía), Government of Costa Rica: INCOPECA-CPI-003-12-2018, R-070-2018-OT-CONAGEBIO, SINAC-CUSBSE-PI-R-032-2018, SINAC-SE-CUS-PI-R-035-2017. DNA sequencing for this project was authorized by the Contract for the Grant of Prior Informed Consent between MINAE-SINAC-ACMC and Jorge Cortés Nuñez for the Basic Research Project: “FK190106—Cuantificación de los vínculos biológicos, químicos y físicos entre las comunidades quimiosintéticas con el mar profundo circundante.” The type specimens were collected during AT42-03 using a suction sampler or the Bushmaster Jr. device, a hydraulically operated net that envelops a cluster of vestimentiferan tubeworms to minimize the loss of associated fauna (Bergquist *et al.* 2002). Specimens were maintained in chilled seawater and photographed using a handheld camera (e.g. Nikon D70, Canon EOS M5). Tissue subsamples were preserved in 95% ethanol for genetic analysis. Specimens were fixed in 10% seawater formalin or 95% ethanol for at least 24 hours, rinsed with fresh water, and transferred to 50% ethanol for long-term archival. Some voucher specimens were frozen at -20 °C for several years prior to fluid preservation. Measurements were made with digital calipers to the nearest 0.1 mm. Methods and terminology follow Anderson (1994, 1995). Specimens were radiographed for meristics.

Additionally, one specimen (SIO 20-41; Fig. 1–2) was micro-CT scanned at California State Polytechnic University, Humboldt using a Nikon XTH 225 scanner. A scan of the entire fish at 100 kV and 185 µA produced 4,476 projections reconstructed into a stack of 2,495 images at a voxel size of 39.37 µm. The image stack and STL file of the skeleton rendering can be found on MorphoSource (morphosource.org) as Project Number 000435550. A more targeted, higher resolution scan of the head at 80 kV and 242 µA produced 3,384 projections at a voxel size of 12.28 µm.

Abbreviations in the text are: HL, head length; SL, standard length. Holotype and paratypes are deposited at the Scripps Institution of Oceanography Marine Vertebrate Collection, La Jolla, USA (SIO), the Museo de Zoología, Universidad de Costa Rica, San José, Costa Rica (MZUCR), and the Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark (ZMUC).

DNA extraction and sequencing were performed by the laboratory of G. Rouse at SIO. Genomic DNA was extracted following the manufacturer’s protocol for commercial kits such as the DNeasy Tissue Kit (Qiagen) or the

Quick-DNA Miniprep, Microprep Plus, or 96 Plus Kit (Zymo Research, Irvine, CA and Tustin, CA). Polymerase chain reaction (PCR) amplification of mitochondrial cytochrome c oxidase subunit I (COI) was performed using the primer pair LCO1490/HCO2198 (Folmer *et al.* 1994). A typical PCR included 1 µl of each primer (10 µM), 2 µl of genomic DNA, and the appropriate concentration of a commercially available reagents such as Apex 2.0x Taq Red DNA Polymerase Master Mix (Genesee Scientific) or Conquest PCR Master Mix (Lambda Biotech, St. Louis, MO). The reaction profile was 94 °C/180s—(94 °C/30s—47 °C/45s—72 °C/60s) \* 5 cycles—(94 °C/30s—52 °C/45s—72 °C/60s) \* 30 cycles—72 °C/300s. PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, OH). Sanger sequencing was performed by Eurofins Genomics (Louisville, KY) or Retrogen, Inc. (San Diego, CA). Consensus sequences were assembled using Geneious (e.g. v.11.1.5). Sequences were deposited into GenBank (accession numbers: OP234394–OP234395).

## Results

### *Pyrolycus jaco* Frable, Seid, Bronson & Møller, sp. nov.

(Figures 1–4, Table 1)

LSIDurn:lsid:zoobank.org:act:1EF499D0-6042-41A5-8E85-0B35A087CBB2

*Pachycara* sp.—Levin *et al.*, 2012: Fig. 2d.

**Holotype:** SIO 20-41 (ex SIO-BIC BI1339), 107+ mm SL, Costa Rica, Jacó Scar, 9°7.041' N 84°50.375' W, 1795 m depth, 18 Oct 2018, HOV *Alvin* dive 4972, suction sampler, G. Rouse and A. Hiley.

**Paratypes:** SIO 20-42 (ex SIO-BIC BI1661), 95+ mm SL (in two pieces), Costa Rica, Jacó Scar, 9°7.050' N 84°50.438' W, 1768 m depth, 04 Nov 2018, HOV *Alvin* dive 4989, suction sampler, L. Levin and D. Casagrande; MZUCR 3319 (ex SIO 20-43; ex SIO-BIC BI1662), 90+ mm SL, Costa Rica, Jacó Scar, 9°7.071' N 84°50.445' W, 1746 m depth, 17 Oct 2018, HOV *Alvin* dive 4971, “Bushmaster” device (in tubeworm mass), E. Cordes and R. Rutstein; ZMUC P2397865 (ex SIO 20-44; ex SIO-BIC BI1663), 72+ mm SL, Costa Rica, Jacó Scar, 9°7.067' N 84°50.367' W, 1785 m depth, 04 Nov 2018, HOV *Alvin* dive 4989, suction sampler, L. Levin and D. Casagrande.

**Diagnosis.** A species of *Pyrolycus* differentiated from its congeners with the following combination of characters: five suborbital bones (vs. six) with 5 pores, occipital pores 2, postorbital pores 3, vertebrae 23 + ~57 = ~80, vomerine and palatine teeth present, total gill rakers 2–3+13–15= 16–17, pectoral fin rays 14–15, upper jaw short 33.9–42.4% HL and snout short 21.3–24.3% HL. It is specifically separated from *Pyrolycus moelleri* in having fewer precaudal vertebrae and total vertebrae, palatine teeth present (vs. absent), three postorbital pores (vs. two) and 14–15 pectoral-fin rays (vs. 13–14). And from *P. manusanus* by having two occipital pores (1-0-1 vs. one, 0-1-0), more gill rakers, fewer vomerine teeth, more palatine teeth, fewer pectoral-fin rays, a larger eye diameter, and a narrower gill slit.

**Description.** Counts and morphometrics are presented in Table 1. Body and head gelatinous; body elongate and compressed posteriorly. Scales and lateral line absent. Peritoneum dark. Head slightly compressed and ovoid; snout rounded and blunt, not extending beyond upper jaw. Eyes round to slightly ovoid and small, not in dorsal profile of head; interorbital space flat to slightly concave, half snout length; nostrils tubular, ca. 5.6 times in snout length. Gill slit long extending ventrally to or just above pectoral-fin base.

Mouth terminal to slightly oblique, posterior edge of maxilla reaching mid-orbit; lips on upper and lower jaw moderately thick. Oral valve weak. Teeth simple and conical. Premaxilla teeth in 2 rows near symphysis becoming a single row posteriorly; 14 teeth in outer row with 5–6 in inner row; outer teeth longer with very slight recurve, those near symphysis longest and more fang-like. Dentary teeth in 2 rows near symphysis becoming single row posteriorly; 15 teeth in outer row with 5–6 in inner row; outer row teeth longer and slightly recurved. Vomerine teeth slightly recurved, in cluster with 7–8 teeth arranged in wedge; palatine teeth simple, 12–15 teeth in single row. Gill rakers short and triangular. Pseudobranch filaments 5. Pyloric caeca present as two nubs.

Cephalic lateralis pores large and round. Nasal pores 2, anteromedial and posteromedial to nostril tube. Suborbital pores 5, arranged in reversed L-shape. Postorbital pores 3 (1, 3 and 4). Preoperculo-mandibular pores 8, 4 from dentary, 1 from angulo-articular and 3 from preopercle. Interorbital pore absent. Occipital pores 2 (1-0-1), on either side, smaller than other pores (Fig. 3).

**TABLE 1.** Morphometric and meristic data for the species of *Pyrolycys*. Data obtained during this study or compiled from literature, as indicated.

Catalog number	<i>Pyrolycys jaco</i> sp. nov.		<i>Pyrolycys manusanus</i>		<i>Pyrolycys moelleri</i>
	SIO 20-41 Holotype	SIO 20-42 Paratype	MZUCR 3319 Paratype	ZMUC 2397865 Paratype	Machida & Hashimoto 2002 Anderson 2006
Standard length	107+	95+	95+	69+	44-156 117-239
Total length	107+	95+	95+	69+	
Meristics					
Dorsal-fin rays	57+	46+	37+	-	73-77 87-88
Anal-fin rays	43+	37+	22+	-	58-60 68-69
Caudal-fin rays	-	-	-	-	11-12 10
Pectoral-fin rays	15/15	15/15	14/14	15/15	16-17 13-14
Pelvic-fin rays	2	2	2	2	2-3 2
Precaudal vertebrae	23	23	23	22	22-23 28
Caudal vertebrae	41+~16	38+	20+	35+	56-59 67-68
Total vertebrae	64+~16	60+	43+	57+	78-81 95-96
Premaxillary teeth rows	2	2	2	2	2
Teeth on premaxilla	14+6	14+5	14+6	14+5	
Vomerine teeth	7	8	7	7	10-12 3-5
Palatine teeth rows	1	1	1	1	absent
Palatine teeth	15/14	13/14	14/14	12	5-6 absent
Dentary teeth rows	2	2	2	2	
Dentary teeth	16+4	16+4	16+5	16+5	3-4
Gill rakers	2+15	2+14/15	2+15	3+13	2+12-14 2+12-15
Branchiostegal rays	6	6	6	6	6
Pseudobranchs	-	-	-	5	2 2-3
Nasal pores	2	-	-	2	2
Postorbital pores	3	-	-	3	2
Suborbital pores	5	-	-	5	6-7 6
Preoperculo-mandibular pores	8	-	8	8	8
interorbital pores	0	-	-	0	0
Occipital pores	2	-	-	2	1 0

.....continued on the next page

TABLE 1. (Continued)

Catalog number	<i>Pyrolycus jaco</i> sp. nov.		<i>Pyrolycus manusanus</i>		<i>Pyrolycus moelleri</i>
	SIO 20-41 Holotype	SIO 20-42 Paratype	MZUCR 3319 Paratype	ZMUC 2397865 Paratype	Machida & Hashimoto 2002 Anderson 2006
Head length, mm	22.1	25.3	27.7	18.1	
% HL					
Head width	39.0	34.7	44.7	34.1	47.4–49.2
Head depth	48.3	45.8	46.7	46.1	52.1–54.5
Upper jaw length	33.9	36.8	42.4	35.4	37.8–42.3
Snout length	22.1	21.3	21.5	24.3	25.8–27.5
Nostril tube length	5.5	5.3	5.2	4.9	
Eye diameter	13.5	12.5	12.1	14.9	10.0–15.3
Gill slit length	26.9	28.1	34.7	32.7	27.2–35.6
Interorbital width	7.5	7.5	7.3	7.0	9.8–10.5
Fleshy interorbital width	13.7	11.7	15.3	13.2	
Interpupillary width	13.6	12.7	17.7	15.0	27.4–28.9
Opercular lobe	8.1	6.7	7.4	7.1	
Body depth	55.9	53.7	49.7	–	
Body depth at anus	49.8	–	46.2	41.9	
Predorsal length	134.6	125.3	110.0	–	
Preanal length	234.1	–	219.0	–	
Prepelvic length	83.9	75.8	75.8	–	
Dorsal-fin base	–	–	–	–	
Anal-fin base	–	–	–	–	
Caudal-fin length	–	–	–	–	13.7–16.3
Pectoral-fin length	53.5	55.1	59.5	60.1	57.5–63.4
Pectoral-fin base depth	20.2	20.4	21.1	–	
Pectoral-fin base/length ratio	37.7	37.1	35.5	34.3	31.3–34.0
Pelvic fin length	9.9	broken	9.1	13.7	missing

Dorsal fin origin on a vertical just before middle of pectoral fin, associated with vertebra five, first two dorsal pterygiophores between vertebra four and five. Anal fin origin before midbody, below first caudal vertebra, first three pterygiophores inserted anterior to first caudal vertebra haemal spine. Pectoral fin origin near body midline; pectoral fins rounded, reaching vertical through 7th–8th dorsal-fin ray. Pelvic fin short and reduced, just anterior pectoral-fin base. Caudal fin destroyed in all specimens.

Neurocranium long. Parasphenoid wing low, at ventral margin of trigeminal foramen but with anteriodorsal projection to just below mid-level of foramen (Fig. 2A). Suborbital bones 5, thin and widely spaced, arranged in reversed L-shape (Fig. 2B). Palatopterygoid series weak, ectopterygoid and mesopterygoid overlapping less than half dorsal and anterior surfaces of quadrate. Metapterygoid reduced and weak.

**Coloration in life (Fig. 1A, 4).** Body light pink to pink lavender, semi-translucent; gut slightly darker than trunk. Top and sides of head somewhat translucent and dark violet, snout pink, anterior of snout and jaw reddish pink. Eyes dark blue-black. Fins light, pale lavender to pinkish, semi-translucent. Faint brownish mottling on body, head and pectoral fins.

**Coloration in preservation (Fig. 1B).** Holotype uniformly tan, body semi-translucent. Fins tan with slight mottling on pectoral fins. Paratypes in 95% ethanol, light tan with very faint mottling body, head and fins (especially SIO 20-42).



**FIGURE 1.** Holotype of *Pyrolycus jaco* sp. nov., SIO 20-41, 107+ mm SL, Jacó Scar, Costa Rica A) freshly collected; B) in preservation, note caudal region removed by collectors; C) superimposition of radiograph over fresh image to estimate vertebral count. Scale bar= 20 mm.

**Etymology.** Named for the type locality and only known habitat, the Jacó Scar site on the Pacific Costa Rica margin, which itself is named in honor of the nearby coastal district of Jacó, Puntarenas, Costa Rica. Name treated as an appositional noun.

**Habitat and distribution.** Specimens were collected or observed in association with colonies of the tubeworms *Lamellibrachia barhami* and *Escarpia spicata* at depths of 1604–1854 m exclusively at Jacó Scar.

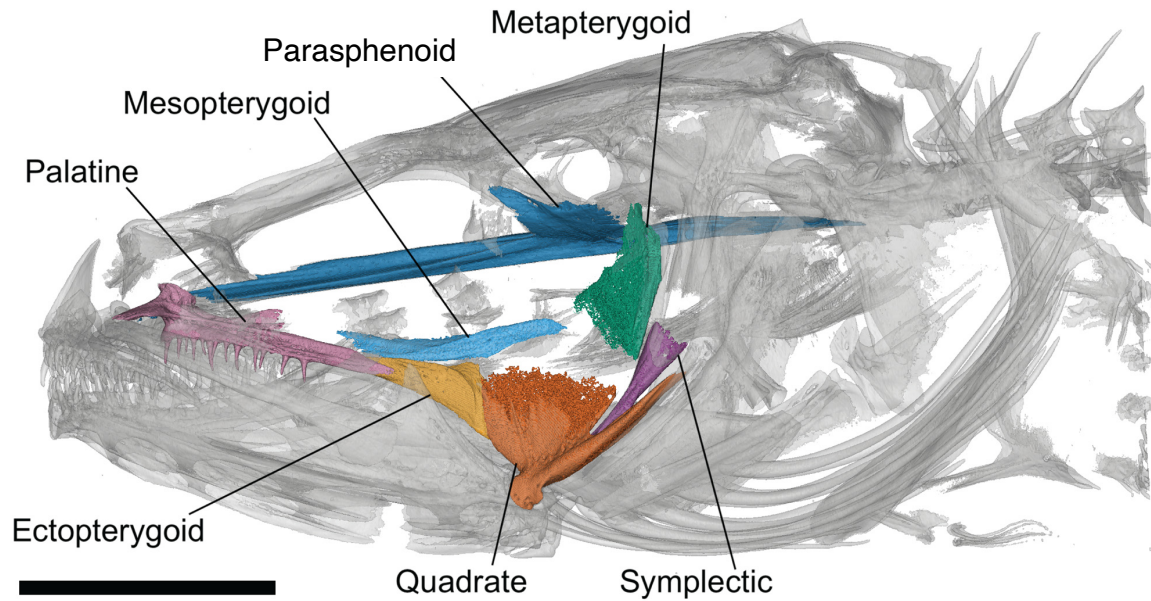
**Diet.** The new species feeds, at least in part, on benthic sessile invertebrates associated with the hydrothermal



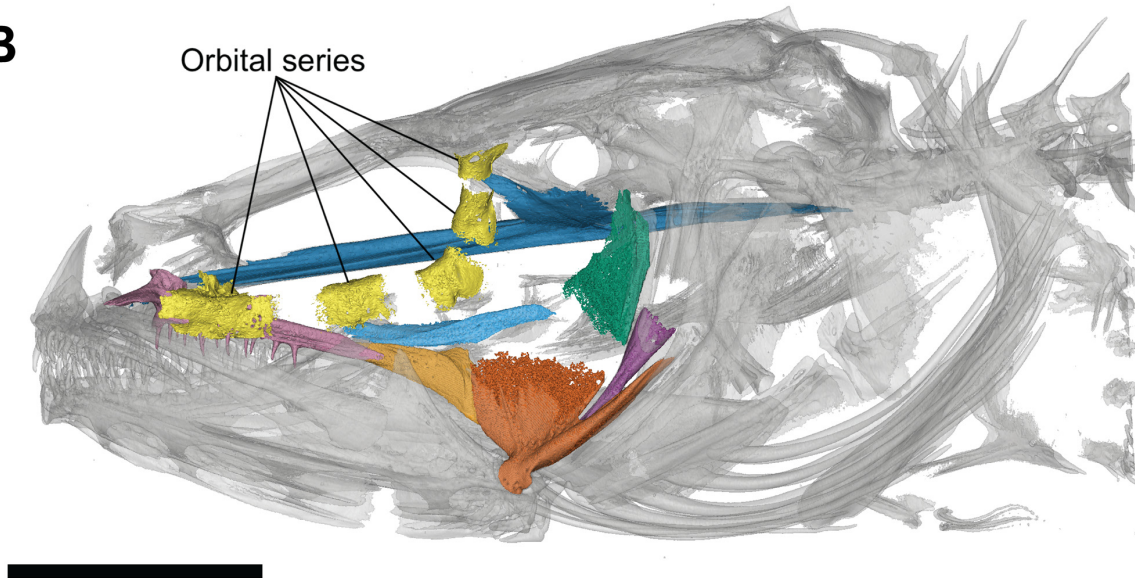
seep area. Dissection of SIO 20-42, radiographs and CT scans reveal shells of *Lepetodrilus* sp. McLean, 1988 and other limpets, as well as a snail in the gut of these specimens.

**Comparisons.** The new species is readily differentiated from congeners in having five suborbital bones (vs. six in the other two species), shorter snout (21.3–24.3 vs. 25.8–29.3% HL) and shorter upper jaw (33.9–42.4 vs. 37.8–42.3% HL). It is further separated from *Pyrolycus moelleri* in having fewer precaudal vertebrae (22–23 vs. 28) and total vertebrae (~80 vs. 95–96), palatine teeth present (vs. absent; Fig. 2A), three postorbital pores (vs. two; Fig. 3) and 14–15 pectoral-fin rays (vs. 13–14). It is differentiated from *P. manusanus* by having two occipital pores (1-0-1 vs. one, 0-1-0; Fig. 3), more gill rakers (2–3+13–15= 16–17 vs. 1–2+12–14=13–15), fewer vomerine teeth (7–8 vs. 10–12), more palatine teeth (12–15 vs. 5–6), fewer pectoral-fin rays (14–15 vs. 16–17), larger eye diameter (12.1–14.9 vs. 8.3% HL), and a narrower gill slit (26.9–34.7 vs. 41.7% HL).

**A**



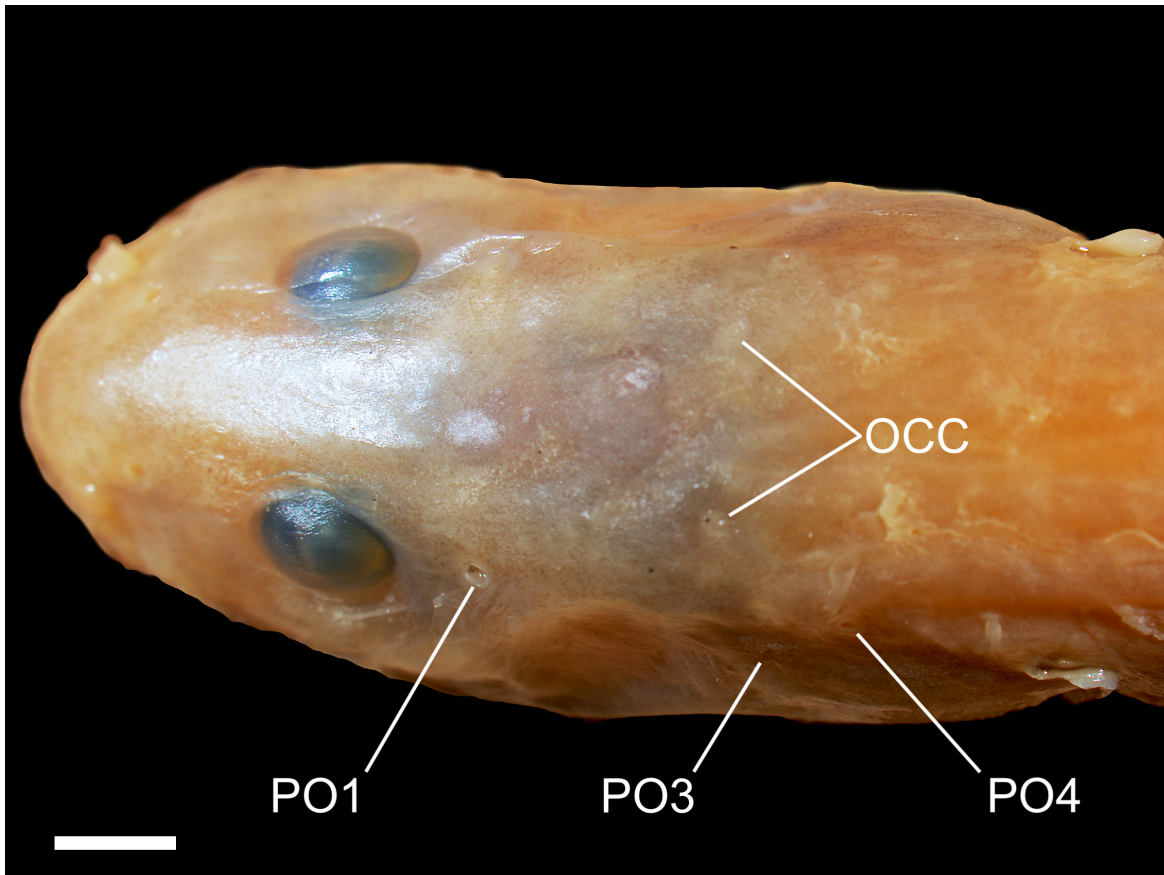
**B**



**FIGURE 2.** CT-image of the lateral left side of the head of SIO 20-41, holotype, *Pyrolycus jaco* sp. nov., 107+ mm SL, with A) parasphenoid and palatopterygoid series and B) orbital series highlighted. Scale bar = 5 mm.

The new species is readily separated from the other two thermophilic genera, *Thermarces* and *Pachycara*. It is differentiated from all three species of *Thermarces* by having a weakly-developed palatopterygoid series (vs. well-developed; Fig. 2A), five suborbital bones and pores (vs. six; Fig. 2B), pelvic bones and fin-rays present (vs. absent), occipital pores present (vs. absent), and fewer precaudal and total vertebrae (22–23 vs. 29–32; ~80 vs. 92–97, respectively).

*Pyrolycus jaco* **sp. nov.** is distinct from members of *Pachycara* by the absence of scales (except present in *P. shcherbachevi* Anderson, 1989 (Møller 2003) from the northern Indian Ocean and *P. alepidotum* Anderson & Mincarone, 2006 and *P. matallanasi* Corbella & Møller, 2014) and a lateral line and in having fewer total vertebrae (~80 vs. 92–125), 2 occipital pores (vs. 0–1), a low parasphenoid wing (vs. high; Fig. 2A), and a weak palatopterygoid series (vs. well-developed).



**FIGURE 3.** Pore configuration on the dorsal surface of the head in *Pyrolycus jaco* **sp. nov.**, holotype, SIO 20-41, 107+ mm SL. PO: postorbital pores; OCC: occipital pores. Scale bar = 2 mm.

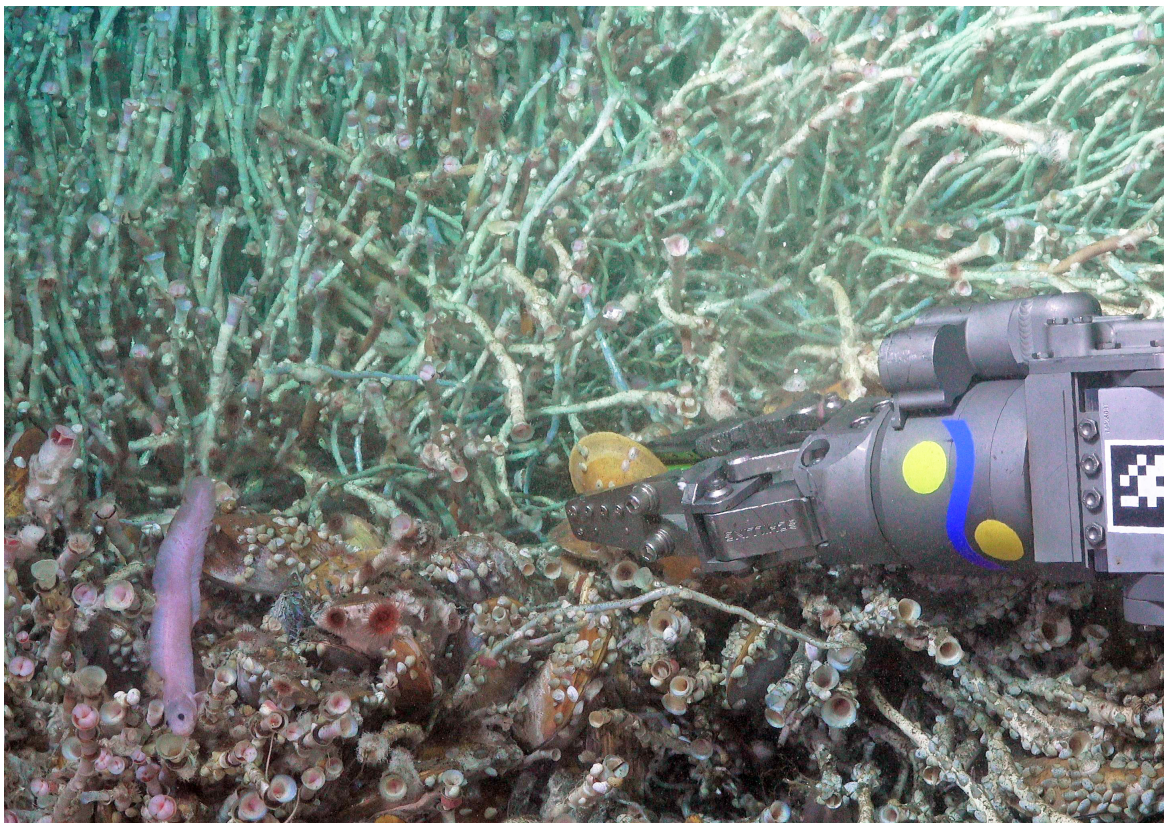
Finally, *Pyrolycus jaco* **sp. nov.** can be compared to the genus *Dieidolycus* Anderson, 1988 in sharing characters such as five suborbital bones, three postorbital pores, and 22–23 precaudal vertebrae, but is readily differentiated by having a low parasphenoid wing (vs. high in *Dieidolycus*), 14–15 pectoral-fin rays (vs. 16–17), and two occipital pores (vs. 0).

The COI sequence for the holotype (609 bp; SIO 20-41; GenBank: OP234394) was 99.84% identical to that of the tissue sample reported in Levin *et al.* (2012; GenBank: OP234395; voucher not located; tissue catalogued as SIO 22-90 [ex SIO BIC BI1642]). BLAST results revealed that there are no other highly similar (>98%) sequences in GenBank, with the most similar being 95.4% for *Lycenchelys tristichodon* DeWitt & Hureau, 1980 (GenBank: HQ713043.1, HQ713045.1 and KX676058.1) and 95.2% for *Pachycara angeloi* Thiel, Knebelberger, Kihara & Gerdes, 2021 (GenBank: MW888715.1). These results demonstrate that the new species is most closely related to other lycodine genera. However, there are not enough available sequences of lycodine species to evaluate the molecular phylogenetic relationships of this species and much of this group in general.



## Discussion

*Pyrolycus jaco* **sp. nov.** is the first vertebrate species to be described from the Jacó Scar hydrothermal seep site, in addition to 21 invertebrate species newly described from this site (Aguado & Rouse 2011; Borda *et al.* 2013; Hatch *et al.* 2020; Lindgren *et al.* 2019; Martin *et al.* 2018; McCowin *et al.* 2020; Pearson & Rouse 2022; Rouse & Kupriyanova 2021; Rouse *et al.* 2018; Salazar-Vallejo 2020; Summers *et al.* 2014; Watson *et al.* 2016), illustrating the extent of novel biodiversity at the Costa Rica methane seeps. It is also the first representative of *Pyrolycus* in the eastern Pacific Ocean. Based on the available material and review of dive footage and images (*e.g.*, Fig. 4), this species is relatively diminutive with individuals estimated to generally be smaller than 150 mm TL. This species and genus as a whole may exhibit characteristics consistent with the process of miniaturization that causes size reduction along with paedomorphic traits such as a reduction of latero-sensory canals, fin rays and scales, and a reduction or simplification of the skeleton (Weitzman and Vari 1988; Britz *et al.* 2009; de Melo *et al.* 2022). *Pyrolycus jaco* **sp. nov.** has a reduced suborbital series relative to the other two species and all three species have generally reduced cranial bones and lack a lateral-line and scales, noted as being potentially paedomorphic states by Anderson (1994). However, these levels of reduction are not as extensive as in well-known taxa (Weitzman and Vari 1988; Britz *et al.* 2009) and further research and additional samples are necessary to explore this. Some aspect of their unique habitats on hydrothermal seeps or vents may contribute to the evolution of these traits, such as reduced prey, complex structure or isolation in relatively small areas. The discovery of this species from a single unique site highlights the possibility of additional highly-adapted zoarcids living in uncharacterized or undiscovered areas of hydrothermal activity. The high degree of endemism for vent- and seep-associated zoarcids is likely a result of their reproductive biology, with few, large eggs and benthic larvae that do not disperse widely.



**FIGURE 4.** Live images of *Pyrolycus jaco* **sp. nov.**, not collected, living among *Lamellibrachia barhami* and *Escarpia spicata* colonies. Photo credit: ROV *SuBastian*/Schmidt Ocean Institute.

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## References

- Aguado, M.T. & Rouse, G.W. (2011) Nautiliniellidae (Annelida) from Costa Rican cold seeps and a western Pacific hydrothermal vent, with description of four new species. *Systematics and Biodiversity*, 9, 109–131.  
<https://doi.org/10.1080/14772000.2011.569033>
- Anderson, M.E. (1994) Systematics and osteology of the Zoarcidae (Teleostei: Perciformes). *J. L. B. Smith Institute of Ichthyology Ichthyological Bulletin*, 1994, 1–120.
- Anderson, M.E. (1995) The eelpout genera *Lycenchelys* Gill and *Taranetzella* Andriashev (Teleostei: Zoarcidae) in the eastern Pacific, with descriptions of nine new species. *Proceedings of the California Academy of Sciences*, 49, 55–113.
- Anderson, M.E. (2006) Studies on the Zoarcidae (Teleostei: Perciformes) of the Southern Hemisphere. XI. A new species of *Pyrolycus* from the Kermadec Ridge. *Journal of the Royal Society of New Zealand*, 36, 63–68.  
<https://doi.org/10.1080/03014223.2006.9517800>
- Bergquist, D.C., Urcuyo, I.A. & Fisher, C.R. (2002) Establishment and persistence of seep vestimentiferan aggregations on the upper Louisiana slope of the Gulf of Mexico. *Marine Ecology Progress Series*, 241, 89–98.  
<https://doi.org/10.3354/meps241089>
- Bohrmann, G., Heeschen, K., Jung, C., Weinrebe, W., Baranov, B., Cailleau, B., Heath, R., Hühnerbach, V., Hort, M., Masson, D. & Trummer, I. (2002) Widespread fluid expulsion along the seafloor of the Costa Rica convergent margin. *Terra Nova*, 14, 69–79.  
<https://doi.org/10.1046/j.1365-3121.2002.00400.x>
- Borda, E., Kudenov, J.D., Bienhold, C. & Rouse, G.W. (2012) Towards a revised Amphinomidae (Annelida, Amphinomida): Description and affinities of a new genus and species from the Nile Deep-sea Fan, Mediterranean Sea. *Zoologica Scripta*, 41, 307–325.  
<https://doi.org/10.1111/j.1463-6409.2012.00529.x>
- Britz, R., Conway, K.W. & Rüber, L. (2009) Spectacular morphological novelty in a miniature cyprinid fish, *Danionella dracula* n. sp. *Proceedings of the Royal Society B: Biological Sciences*, 276, 2179–2186.  
<https://doi.org/10.1098/rspb.2009.0141>
- de Melo, M.R.S., Gomes, A.A., Møller, P.R. & Nielsen, J.G. (2022) A new species of the rare deep-sea genus *Sciadonus* Garman, 1899 (Teleostei, Bythitidae) from off Brazil, with a discussion of the evolution of troglomorphy and miniaturization in the aphyonid clade. *Deep Sea Research Part I: Oceanographic Research Papers*, 180, 103684.  
<https://doi.org/10.1016/j.dsr.2021.103684>
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Fricke, R., Eschmeyer, W.N. & Van der Laan, R. (Eds.) (2022) Eschmeyer's Catalog of Fishes: genera, species, references. Available from: <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp> (accessed 18 July 2022)
- Hatch, A.S., Liew, H., Hourdez, S. & Rouse, G.W. (2020) Hungry scale worms phylogenetics of *Peinaleopolynoe* (Polynoidae, Annelida), with four new species. *ZooKeys*, 2020, 27–74.  
<https://doi.org/10.3897/zookeys.932.48532>
- Levin, L.A., Orphan, V.J., Rouse, G.W., Rathburn, A.E., Ussler, W., Cook, G.S., Goffredi, S.K., Perez, E.M., Waren, A., Grupe, B.M., Chadwick, G. & Strickrott, B. (2012) A hydrothermal seep on the Costa Rica margin: Middle ground in a continuum of reducing ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 279, 2580–2588.  
<https://doi.org/10.1098/rspb.2012.0205>
- Lindgren, J., Hatch, A.S., Hourdez, S., Seid, C.A. & Rouse, G.W. (2019) Phylogeny and biogeography of *Branchipolynoe* (Polynoidae, Phyllococida, Aciculata, Annelida), with descriptions of five new species from methane seeps and hydrothermal vents. *Diversity*, 11, 153.  
<https://doi.org/10.3390/d11090153>
- Machida, Y. & Hashimoto, J. (2002) *Pyrolycus manusanus*, a new genus and species of deep-sea eelpout from a hydrothermal

- vent field in the Manus Basin, Papua New Guinea (Zoarcidae, Lycodinae). *Ichthyological Research*, 49, 1–6.
- Martin, J.W., Wall, A.R., Shank, T., Cha, H., Seid, C.A. & Rouse, G.W. (2018) A new species of *Alvinocaris* (Crustacea: Decapoda: Caridea: Alvinocarididae) from Costa Rican methane seeps. *Zootaxa*, 4504 (3), 418–430. <https://doi.org/10.11646/zootaxa.4504.3.7>
- Mau, S., Rehder, G., Sahling, H., Schleicher, T. & Linke, P. (2014) Seepage of methane at Jaco Scar, a slide caused by seamount subduction offshore Costa Rica. *International Journal of Earth Sciences*, 103, 1801–1815. <https://doi.org/10.1007/s00531-012-0822-z>
- McCowin, M.F., Feehery, C. & Rouse, G.W. (2020) Spanning the depths or depth-restricted: Three new species of *Bathymodiolus* (Bivalvia, Mytilidae) and a new record for the hydrothermal vent *Bathymodiolus thermophilus* at methane seeps along the Costa Rica margin. *Deep-Sea Research Part I: Oceanographic Research Papers*, 164, 103322. <https://doi.org/10.1016/j.dsr.2020.103322>
- Møller, P.R. (2003) Two new species of *Pachycara* Zugmayer (Teleostei: Zoarcidae) from the Indian ocean, with redescription of *Pachycara shcherbachevi* Anderson. *Copeia*, 2003 (2), 357–365. [https://doi.org/10.1643/0045-8511\(2003\)003\[0357:TNSOPZ\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2003)003[0357:TNSOPZ]2.0.CO;2)
- Pearson, K.A.M. & Rouse, G.W. (2022) Vampire Worms; A revision of *Galapagomystides* (Phyllodocidae, Annelida), with the description of three new species. *Zootaxa*, 5128 (4), 451–485. <https://doi.org/10.11646/zootaxa.5128.4.1>
- Priede, I.G. (2017) *Deep-Sea Fishes: Biology, Diversity, Ecology and Fisheries*. Cambridge University Press, Cambridge, 504 pp. <https://doi.org/10.1017/9781316018330>
- Rouse, G.W., Carvajal, J.I. & Pleijel, F. (2018) Phylogeny of Hesionidae (Aciculata, Annelida), with four new species from deep-sea eastern Pacific methane seeps, and resolution of the affinity of *Hesiohyra*. *Invertebrate Systematics*, 32, 1050–1068. <https://doi.org/10.1071/IS17092>
- Rouse, G.W. & Kupriyanova, E.K. (2021) *Laminatubus* (Serpulidae, Annelida) from eastern Pacific hydrothermal vents and methane seeps, with description of two new species. *Zootaxa*, 4915 (1), 1–27. <https://doi.org/10.11646/zootaxa.4915.1.1>
- Salazar-Vallejo, S.I. (2020) Four new deep-water flabelligerid species from Pacific Costa Rica (Annelida, Sedentaria, Flabelligeridae). *Zootaxa*, 4885 (4), 560–578. <https://doi.org/10.11646/zootaxa.4885.4.6>
- Summers, M.M., Al-Hakim, I.I. & Rouse, G.W. (2014) Turbo-taxonomy: 21 new species of Myzostomida (Annelida). *Zootaxa*, 3873 (4), 301–344. <https://doi.org/10.11646/zootaxa.3873.4.1>
- Thiel, R., Kneibelsberger, T., Kihara, T. & Gerde, K. (2021) Description of a new eelpout *Pachycara angeloi* sp. nov. (Perciformes: Zoarcidae) from deep-sea hydrothermal vent fields in the Indian Ocean. *Zootaxa*, 4980 (1), 99–112. <https://doi.org/10.11646/zootaxa.4980.1.6>
- Watson, C., Ignacio Carvajal, J., Sergeeva, N.G., Pleijel, F. & Rouse, G.W. (2016) Free-living calamyzin chrysopetalids (Annelida) from methane seeps, anoxic basins, and whale falls. *Zoological Journal of the Linnean Society*, 177, 700–719. <https://doi.org/10.1111/zoj.12390>
- Weitzman, S.H. & Vari, R.P. (1988) Miniaturization in South American freshwater fishes; an overview and discussion. *Proceedings of the Biological Society of Washington*, 101, 444–465.