

Independent radiation of snailfishes into the hadal zone confirmed by *Paraliparis selti* sp. nov. (Perciformes: Liparidae) from the Atacama Trench, SE Pacific

THOMAS D. LINLEY¹, MACKENZIE E. GERRINGER², HEATHER RITCHIE^{3*}, JOHANNA N. J. WESTON¹, AMY SCOTT-MURRAY⁴, VINCENT FERNANDEZ⁴, JHOANN L. CANTO⁵, FRANK WENZHÖFER^{6 7 8}, RONNIE N. GLUD^{6 9 10}, ALAN J. JAMIESON¹¹

Corresponding author: T. Linley, Thomas.Linley@newcastle.ac.uk

Supplementary material

a.1. Spanish language abstract

Resumen

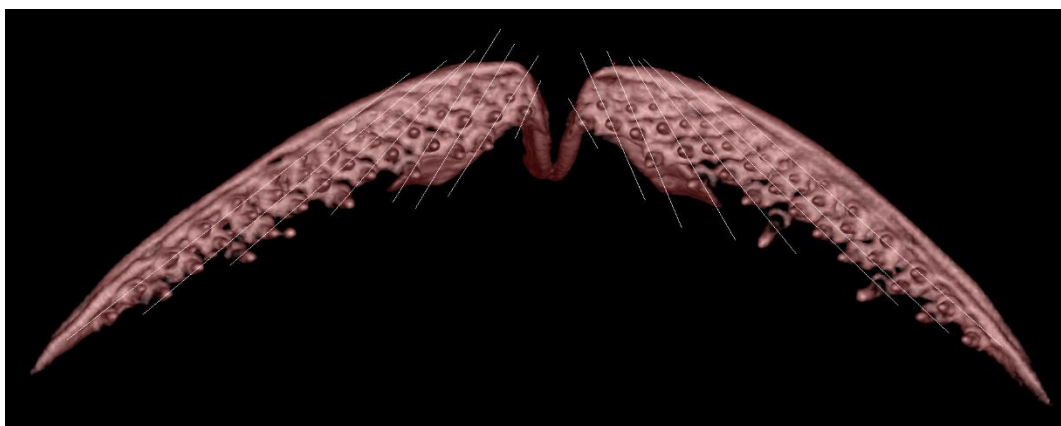
Los liparidos se encuentran entre las familias de peces marinos que han experimentado una rápida radiación, lo que ha resultado en una distribución global que se extiende desde el intermareal costero hasta las profundas fosas de subducción. La real diversidad de los liparidos de aguas profundas, particularmente en las zonas hadal (> 6000 m) del hemisferio sur, sigue siendo incierta debido a la escasez de muestras. A continuación, presentamos la diversidad de liparidos hadal de la Fosa de Atacama, que se extiende a lo largo de la costa suroeste de América del Sur. Utilizando trampas y cámaras con cebo de caída libre, documentamos al menos tres especies de liparidos de la zona hadal, entre 5920 y 7608 m. Se recolectó un espécimen de 6714 m. Describimos a *Paraliparis selti* sp. nov., basados en un enfoque taxonómico morfológico y molecular combinado (16S, COI y Cyt-b). *Paraliparis selti* sp. nov. es morfológicamente distinto de otras especies de liparidos descritos debido a exhiben una combinación de un alto número de vértebras (65), particularmente las vértebras abdominales (12), radios de la aleta dorsal (60), radios de la aleta anal (52) y radios de la aleta caudal (8); número comparativamente bajo de radios de la aleta pectoral (18) que forma una muesca profunda con dos radios no rudimentarios ampliamente espaciados; boca horizontal; mandíbulas simples y no uniseriales; color en vida negro azulado anteriormente que se vuelve pálido hacia la parte posterior del cuerpo, pigmento azul que se vuelve oscuro cuando se procede a su conservación; peritoneo negro. Se utilizó un micro-CT para minimizar la disección del espécimen y proporcionar un holotipo digital de calidad. *Paraliparis selti* sp. nov. destaca la importancia de los Liparidae de profundidades hadal y además proporciona evidencia de una radiación independiente adicional de liparidos en la zona hadal.

Supplementary Table 1: Lander deployments. Date in the format DD/MM/YYYY, depth (m) and temperature (°C) calculated from the lander's pressure-temperature sensor. Latitude and longitude are given in decimal degrees. The number of still images and video clips (30 sec duration) recorded on the bottom and total time on the bottom in minutes are shown.

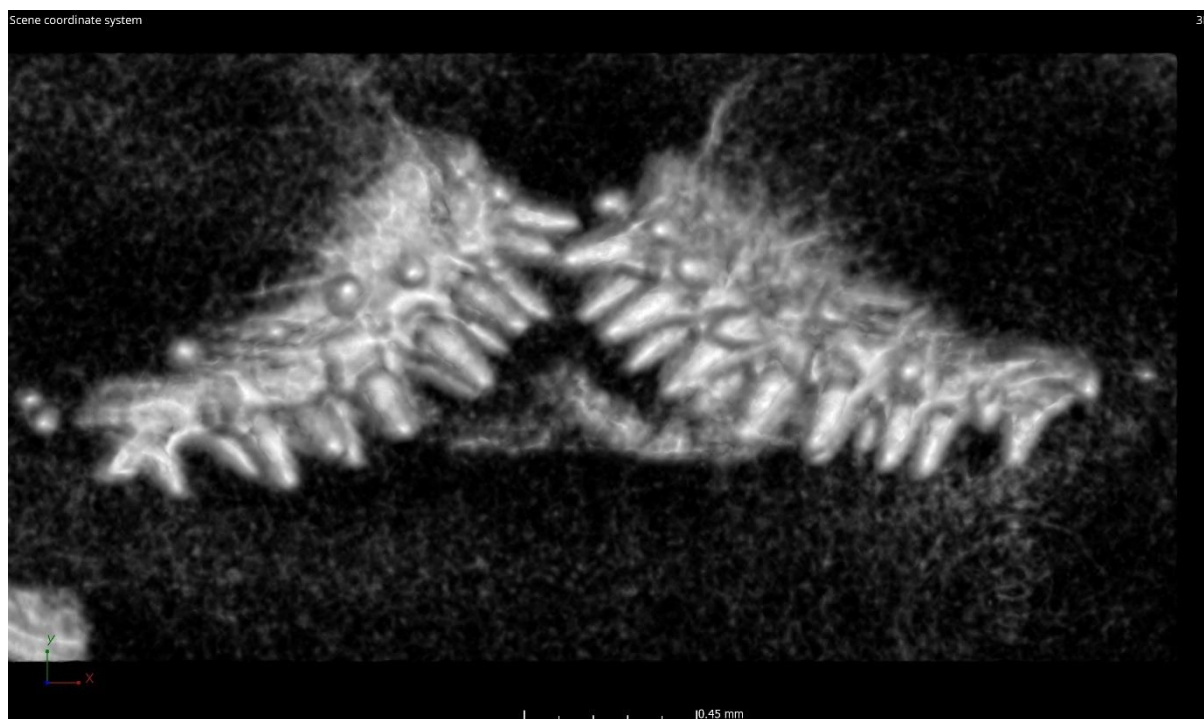
Date	Depth	Temperature	Lat	Long	Stills	Videos	Bottom time
05/03/2018	2537	1.81	-23.82100	-70.83613	383	382	586
07/03/2018	7782	2.21	-24.27500	-71.42313	93	93	1420
07/03/2018	7834	2.21	-24.28000	-71.42315	883	1075	1553
10/03/2018	7608	2.17	-23.82900	-71.34633	1315	1315	2030
10/03/2018	7493	2.16	-23.83300	-71.34392	938	1053	2056
13/03/2018	8052	2.25	-23.37957	-71.34472	12	796	1591
13/03/2018	7204	2.11	-23.37307	-71.39295	1123	1129	1849
16/03/2018	7139	2.09	-23.04997	-71.25073	1062	1059	2346
16/03/2018	6974	2.07	-23.05025	-71.23322	582	581	2387
19/03/2018	5563	1.86	-22.94913	-71.61995	788	788	1216
19/03/2018	4974	1.79	-22.93803	-71.67810	83	84	1008
22/03/2018	6714	2.03	-21.74162	-71.25775	983	979	3238
22/03/2018	6520	2.00	-21.72033	-71.26355	944	942	3341
25/03/2018	5920	1.91	-20.34347	-71.12135	739	739	1096
25/03/2018	6025	1.93	-20.34350	-71.13040	757	756	1123
27/03/2018	4051	1.76	-20.34243	-70.99010	276	276	409
27/03/2018	4100	1.75	-20.34693	-70.99010	304	304	451



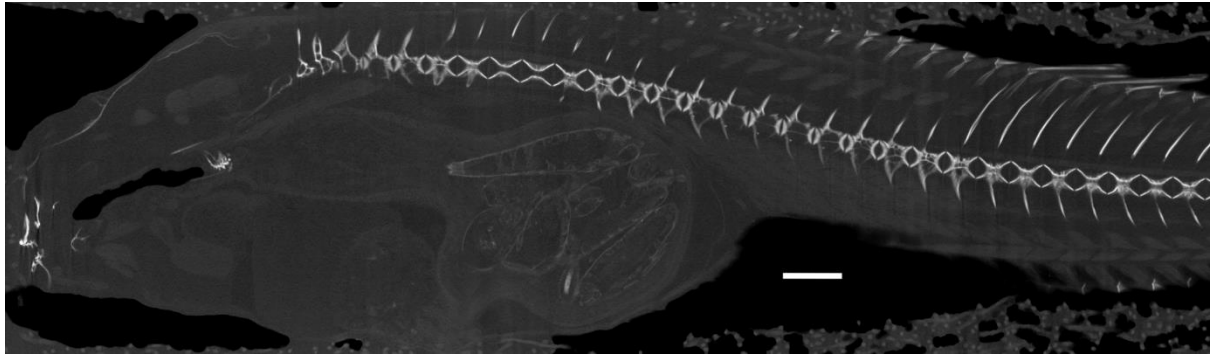
Supplementary Figure 1: High resolution 3D rendering of *Paraliparis selti* **sp. nov.** MNHNC ICT 76227 from CT data. The rendering has been laterally bisected along the midline and only the left side it displayed for greater clarity. Scale at 1.5 mm.



Supplementary Figure 2: 3D rendering of the upper premaxillary dentition in *Paraliparis selti* **sp. nov.** MNHNC ICT 76227 from CT data. Tooth rows as identified by the authors are shown.



Supplementary Figure 3: 3D rendering of the dorsal pharyngeal jaw in *Paraliparis selti* **sp. nov.** MNHNC ICT 76227 from CT data. A ventral jaw is not clearly visible.



Supplementary Figure 4: Longitudinal slice of *Paraliparis selti* **sp. nov.** MNHNC ICT 76227 from CT data showing amphipods up to 7 mm long in the gut. Scale is 2 mm.

a.2. DNA barcoding and phylogenetic analyses

Materials and methods

In addition to the more extensive Liparidae COI dataset, we constructed two additional datasets for all snailfish species with available sequence data at each loci (16S, COI, Cyt-b, and concatenated). The first dataset comprised of 10 Liparidae species with a reduced sequence length: 16S (575 bp), COI (645 bp) and Cyt-b (315 bp) (Supplementary Table 3). A further dataset was compiled to test the effect of sequence length on phylogenetic topologies. While the majority of 16S, COI and Cyt-b sequences available on GenBank are small, partial gene sequences, there are six Liparidae species with longer gene sequences available: 16S (1476 bp), COI (1399 bp) and Cyt-b (1007 bp) (Supplementary Table 4).

Sequences were aligned, and optimal evolutionary models were calculated as per the methods described in the main body of the manuscript. *Apotcycylus ventricosus* was again selected as the outgroup as it is a closely related species. Phylogenetic relationships were also reconstructed using the previously described methods where the optimal evolutionary models used were: the Kimura 2-parameter with gamma distribution (K2+G) for reduced length 16S, the generalised time reversible with gamma with gamma distribution (GTR+G) for longer length 16S, and the Hasegawa, Kishino and Yano model with gamma distribution (HKY+G) for COI and Cyt-b.

Supplementary Table 2: Primer sets used for PCR amplification of three mitochondrial barcoding genes.

Gene	Primer name	Primer sequence	Reference
16S	16S_liparids_F	5'-CTA TTA ATA CCC CCA AAT ACC CC-3'	Gerringer et al. (2017)
	16S_liparids_R	5'-CGA TGT TTT TGG TAA ACA GGC G-3'	Gerringer et al. (2017)
	16S_liparids_R2	5'-GAT TTC ATC AGG TAG GGG GAG GGC-3'	Gerringer et al. (2017)

	16S_liparids_I	5'-CCA AAA ACA TCG CCT CTT GTA CCC-3'	Gerringer et al. (2017)
COI	COI_liparids_F	5'-GCC ATC CTA CCT GTG GCC ATC ACA CG-3'	Gerringer et al. (2017)
	COI_liparids_R	5'-AGT GGG ATA AAA CAA ATG CGG G-3'	Gerringer et al. (2017)
	Liparid_WardsF1	5'-TCG ACT AAT CAC AAA GAC ATT GGC AC-3'	Ward et al. (2005)
	Liparid_WardsR1	5'-TAA ACT TCG GGA TGG CCA AAG AAT CA-3'	Ward et al. (2005)
	COI_liparids_I	5'-CTG ATT CTT TGG CCA TCC CGA AG-3'	Gerringer et al. (2017)
Cyt-b	Cytb_liparids_F	5'-ATG GCA AGC CTA CGA AAA ACC CAC C-3'	Gerringer et al. (2017)
	Cytb_liparids_R	5'-TAT TCT CTA TGA AGC CGG TAA GGG-3'	Gerringer et al. (2017)
	Cytb_liparids_R2	5'-GGG TTA GTT GAG CCT GTT TCG TG-3'	Gerringer et al. (2017)

Supplementary Table 3: Species, sequence accession numbers and references for all samples included in the analysis of *Paraliparis selti* **sp. nov.** with the liparids using shorter sequence lengths: 16S (575 bp), COI (645 bp) and Cyt-b (315 bp). Asterisks indicate unpublished data.

	16S	COI	Cyt-b	Reference
Family: Cyclopteridae				
<i>Aptocyclus ventricosus</i>	AP004443	AP004443	AP004443	Miya et al. (2003)
Family: Liparidae				
<i>Liparis gibbus</i>	EF508352	AM498312	AM498332	Knudsen et al. (2007) Byrkjedal et al. (2007)
<i>Liparis liparis</i>	EF508354	KJ204977	EU492094	Knudsen et al. (2007) Knebelsberger et al. (2014) van Pelt-Heerschap and Stein*
<i>Liparis montagui</i>	KJ128814	KJ128532	EU492339	Bergsten et al.* Noren et al.*
<i>Liparis mucosus</i>	EF458456	JQ354182	DQ082907	Park et al.* Elz et al.* Byrne et al.*
<i>Liparis ochotensis</i>	MG718032	MG718032	MG718032	Sim et al.*
<i>Notoliparis kermadecensis</i>	KY659190	KY659180	KY659197	Gerringer et al. (2017)
<i>Notoliparis stewartia</i>	KY659188	KY659178	KY659196	Gerringer et al. (2017)
<i>Paraliparis selti</i> sp. nov.	MN423252	MN422493	MN422494	This paper
<i>Pseudoliparis belyaevi</i>	-	-	-	T.P. Satoh, Pers. Comm.
<i>Pseudoliparis swirei</i>	KY659194	KY659185	KY659202	Gerringer et al. (2017)

Supplementary Table 4: Species, sequence accession numbers and references for all samples included in the analysis of *Paraliparis selti* **sp. nov.** with the liparids using longer sequence lengths: 16S (1476 bp), COI (1399 bp) and Cyt-b (1007 bp).

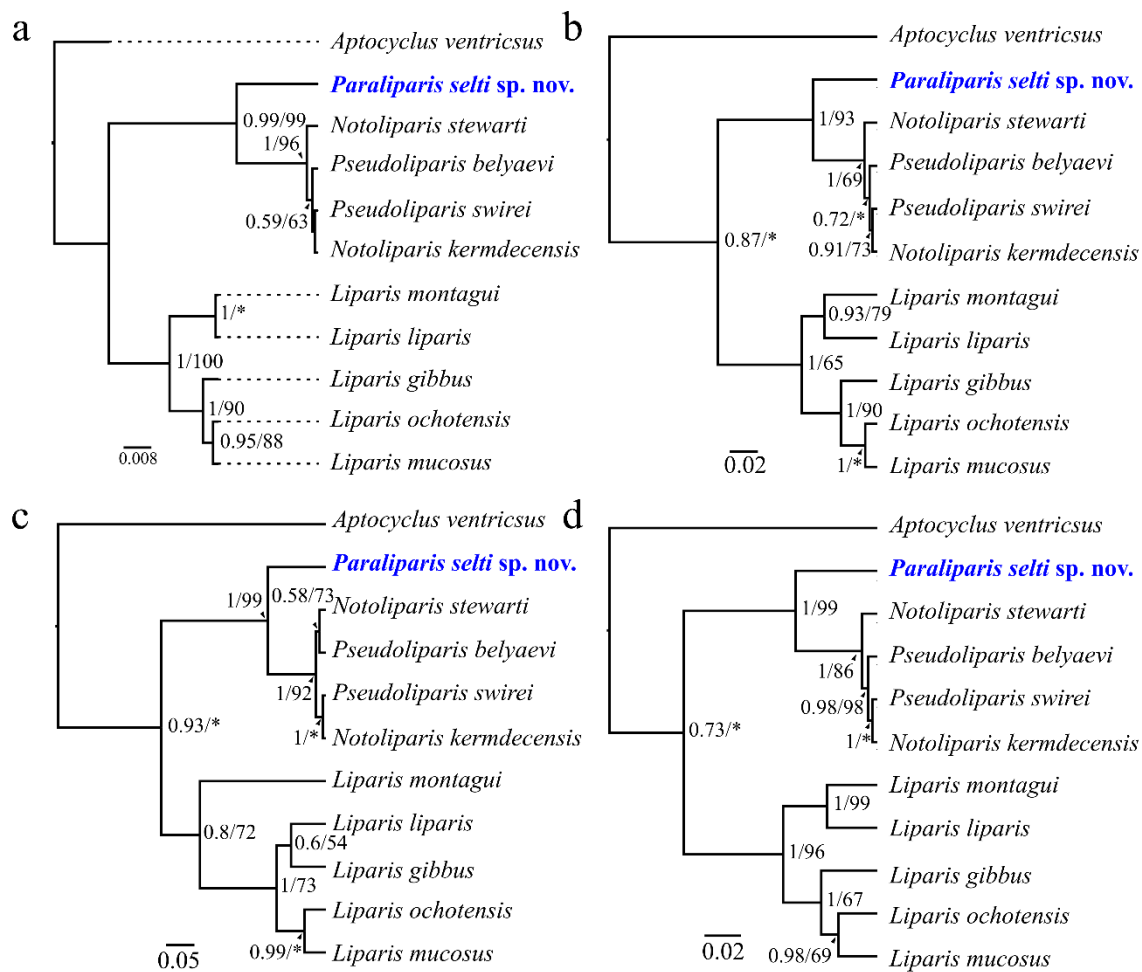
	16S	COI	Cty-b	Reference
Family: Cyclopteridae				
<i>Aptocyclus ventricosus</i>	AP004443	AP004443	AP004443	Miya et al. (2003)
Family: Liparidae				
<i>Liparis ochotensis</i>	MG718032	MG718032	MG718032	Sim et al.*
<i>Notoliparis kermadecensis</i>	KY659190	KY659180	KY659197	Gerringer et al. (2017a)
<i>Notoliparis stewarti</i>	KY659188	KY659178	KY659196	Gerringer et al. (2017a)
<i>Paraliparis selti</i> sp. nov.	MN423252	MN422493	MN422494	This study
<i>Pseudoliparis belyaevi</i>	-	-	-	T.P. Satoh, Pers. Comm.
<i>Pseudoliparis swirei</i>	KY659194	KY659185	KY659202	Gerringer et al. (2017a)

Results

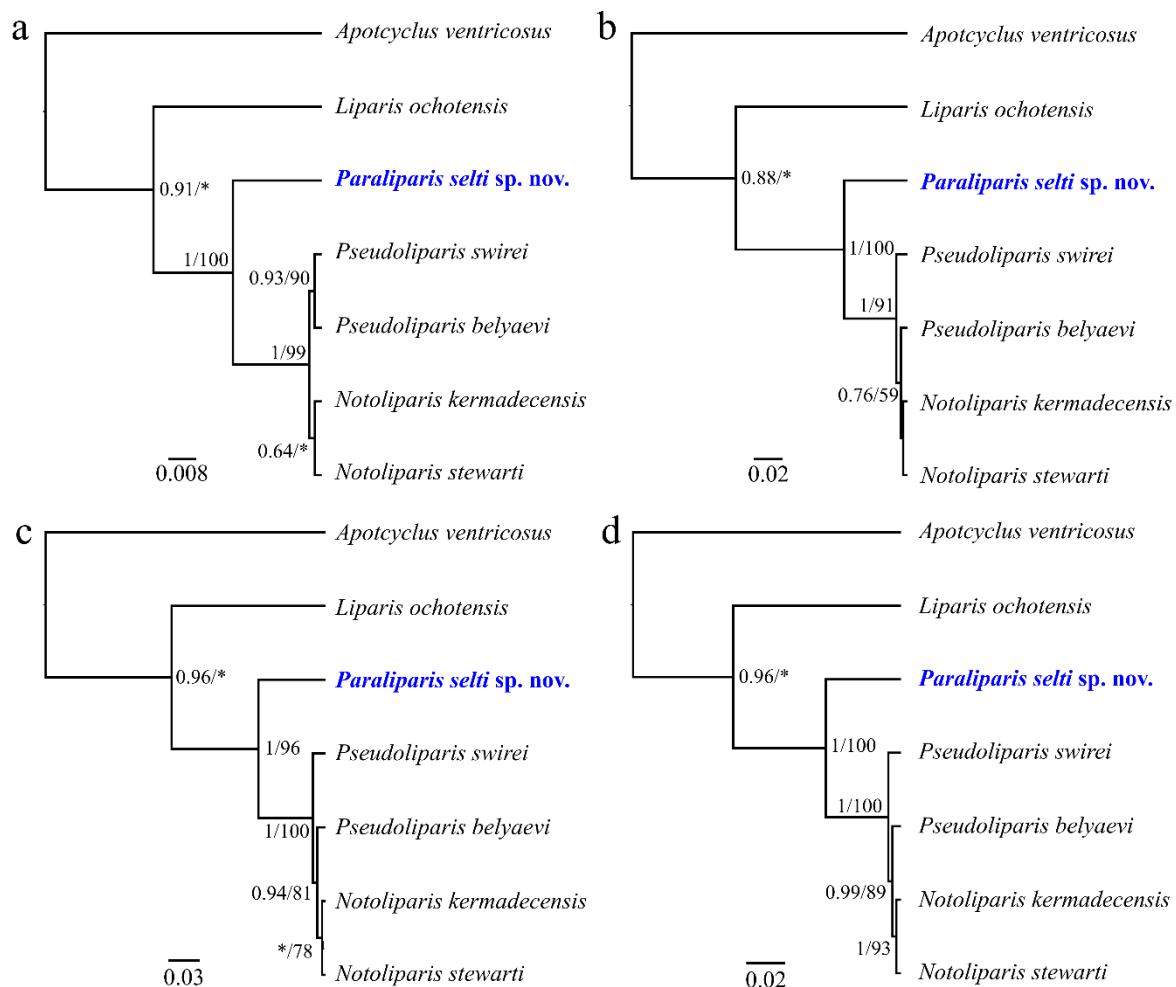
The phylogenetic relationships and placement of *P. selti* **sp. nov.** with other Liparidae species was investigated by comparing all available liparid species with overlapping 16S, COI and Cyt-b sequences that are publicly available within GenBank (Supplementary Table 3 and Supplementary Table 4). The topologies for the reduced length sequences are shown in Supplementary Figure 5 **Error! Reference source not found.** and the longer length sequences are shown in Supplementary Figure 6.

Across all topologies, *P. selti* **sp. nov.** is placed within the liparids, reciprocally monophyletic to the *Liparis* spp. clade and basal to the clade of the four other hadal snailfishes (i.e., *Pseudoliparis swirei*, *P. belyaevi*, *Notoliparis kermadecensis*, and *N. stewarti*). The phylogenetic placement of *P. selti* **sp. nov.** is highly congruent between, and well-supported across, the individual 16S, COI, and Cyt-b topologies and the concatenated dataset.

We found congruence in the placement of *P. selti* **sp. nov.** among the shorter and longer gene sequences topologies (Supplementary Figure 5 and Supplementary Figure 6). Therefore, we consider the shorter gene sequences to represent a useful and accurate way to reconstruct phylogenetic relationships within the Liparidae.



Supplementary Figure 5: Bayesian trees showing the relationship of *Paraliparis selti* sp. nov. within the liparids using shorter sequences lengths: 16S (575 bp), COI (645 bp) and Cyt-b (315 bp). Individual gene topologies are shown for (a) 16S, (b) COI, (c) Cyt-b, and (d) a concatenated dataset. Bayesian posterior probabilities and Maximum-likelihood bootstrap supports are shown on branch nodes. Values less than 50% or values not supported by the alternative method are not stated or depicted by an asterisk.



Supplementary Figure 6: Bayesian trees showing the relationship of *Paraliparis selti* sp. nov. within the liparids using longer sequences lengths: 16S (1476 bp), COI (1399 bp) and Cyt-b (1007 bp). Individual gene topologies are shown for (a) 16S, (b), COI, (c) Cyt-b, and (d) a concatenated dataset. Bayesian posterior probabilities and Maximum-likelihood bootstrap supports are shown on branch nodes. Values less than 50% or values not supported by the alternative method are not stated or depicted by an asterisk.

Supplementary Video 1: “Liparid sp. indet. 2-PCT”, in situ video of Liparid sp. indet. 2-PCT

Supplementary Video 2: “Liparid sp. indet. 3-PCT”, in situ video of Liparid sp. indet. 3-PCT

Supplementary Video 3: “*Paraliparis selti* sp. nov. x2”, in situ video of two *Paraliparis selti* sp. nov.

Supplementary References

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