

Table S1 Haplotype name, collection information, and GenBank accession numbers for *Parborlasia* spp. samples from in South America, Antarctica and the sub-Antarctic Islands. Station numbers 04-xx and 06-xx were collected on the 2004 and 2006 cruises aboard the R/V Lawrence M. Gould, respectively. Others were either obtained from the ICEFISH cruises (via S.J. Lockhart) or directly from Genbank (i.e., the McMurdo Sound sample).

Haplotype	n	Latitude	Longitude	Collection locality and station information	Depth	Accession #	
						COI	16S
A1	2	S63°40.00'	W57°19.75'	AP; Eagle Island - Station 04-40	335 m	EU194802	EU194791
A1	1	S63°23.05'	W60°03.4'	AP; Trinity Peninsula - Station 04-51	277 m	EU194802	EU194791
A1	4	S62°56.599'	W60°39.07'	AP; Deception Island - Station 04-64	161 m	EU194802	EU194791
A1	11	S62°59.2'	W60°39.9'	AP; Deception Island - Station 04-64-b	5 m	EU194791	EU194791
A1	1	S65°39.8'	W68°01.8'	AP; Continental Shelf - Station 04-82	278 m	EU194802	EU194791
A1	1	S67°39.803'	W68°14.695'	AP; Antarctica - Station 06-47	170 m	EU194802	EU194791
A1	1	S66°36.663'	W68°18.533'	AP; Antarctica - Station 06-31	261 m	EU194802	EU194791
A1	6	S65°01.554'	W63°18.232'	AP; Antarctica - Station 06-60	400 m	EU194802	EU194791
A1	3	S68°11.03'	W67°37.12'	AP; Antarctica - Plankton Tow LMG06-37-PT32	200 m	EU194802	EU194791
A1	1	S54°21'	E3°22.8'	BI; Bouvet Island - 71-BT41	200 m	EU194802	EU194791
A1	1	S54°22'	W36°30'	SG; South Georgia Islands - 41-BT22	230 m	EU194802	EU194791
A1 (GenBank)	1	---	---	McS; McMurdo Sound	---	AJ436939	AJ436983

A2	1	S62°59.2'	W60°39.9'	AP; Deception Island - Station 04-64-b	5 m	EU194791	EU194792
A6	1	S65°39.8'	W68°01.8'	AP; Continental Shelf - Station 04-82	278 m	EU194802	EU194794
B1	1	S62°43.535'	W55°11.224'	SG; South Georgia 45- BT24	167 m	EU194812	EU194791
C1	1	S62°56.599'	W60°39.07'	AP; Deception Island - Station 04-64	161 m	EU194805	EU194791
D1	1	S62°56.599'	W60°39.07'	AP; Deception Island - Station 04-64	161 m	EU194804	EU194791
D1	1	S62°34.209'	W60°02.782'	AP; Antarctica - Station 06-24	200 m	EU194804	EU194791
D3	1	S65°01.554'	W63°18.232'	AP; Antarctica - Station 06-60	400 m	EU194804	EU194800
E1	1	S62°59.2'	W60°39.9'	AP; Deception Island - Station 04-64-b	5 m	EU194806	EU194791
F1	1	S63°13.742'	W58°45.328'	AP; Trinity Peninsula - Station 04-49	87 m	EU194803	EU194791
F1	2	S62°59.2'	W60°39.9'	AP; Deception Island - Station 04-64-b	5 m	EU194803	EU194791
F1	2	S65°39.8'	W68°01.8'	AP; Continental Shelf - Station 04-82	278 m	EU194803	EU194791
F1	1	S66°36.663'	W68°18.533'	AP; Antarctica - Station 06-31	261 m	EU194803	EU194791
F1	10	S65°01.554'	W63°18.232'	AP; Antarctica - Station 06-60	400 m	EU194803	EU194791
F1	1	S54°23.83'	E3°28.95'	BI; Bouvet Island - 80- BT47	211 m	EU194803	EU194791
F1	1	S53°55.77'	W37°0.65'	SG; South Georgia - 38- BT18	258 m	EU194803	EU194791
F3	1	S65°55.558'	W66°54.225'	AP; Antarctica - Station 06-53	183 m	EU194803	EU194800

F4	1	S58°55.8'	W26°49'	SSI; South Sandwich Islands - 52-OT43	120 m	EU194803	EU194795
F5	1	S65°37.424'	W67°47.077'	AP; Renaud Island - Station 04-78	217 m	EU194803	EU194793
F9	1	S65°01.554'	W63°18.232'	AP; Antarctica - Station 06-60	400 m	EU194803	EU194801
G1	1	S65°01.554'	W63°18.232'	AP; Antarctica - Station 06-60	400 m	EU194825	EU194791
H1	1	S65°39.8'	W68°01.8'	AP; Continental Shelf - Station 04-82	278 m	EU194808	EU194791
H1	1	S65°39.8'	W68°01.8'	AP; Continental Shelf - Station 04-82	278 m	EU194808	EU194791
I1	1	S62°34.209'	W60°02.782'	AP; Antarctica - Station 06-24	200 m	EU194813	EU194791
J1	1	S53°27.118'	W41°16.92'	SG; Shag Rocks - 29-BT13	200 m	EU194811	EU194796
J1	1	S56°15.06'	W27°33.33'	SSI; South Sandwich Islands - 48-BT27	440 m	EU194811	EU194796
K1	1	S54°21'	E3°22.8'	BI; Bouvet Island - 71-OT46	202 m	EU194810	EU194791
L1	2	S64°24.62'	W64°30.13'	AP; Anvers Island - Station 04-74	202 m	EU194807	EU194791
M1	1	S65°39.8'	W68°01.8'	AP; Continental Shelf - Station 04-82	278 m	EU194809	EU194791
N1	1	S65°01.554'	W63°18.232'	AP; Antarctica - Station 06-60	400 m	EU194826	EU194791
O8	1	S54°49'	W60°16'	SEA; Argentinean Waters - Station 06-6	110 m	EU194817	EU194797
P8	1	S53°47'	W60°42'	SEA; Argentinean Waters - Station 06-4	170 m	EU194816	EU194797
Q8	1	S53°47'	W60°42'	SEA; Argentinean Waters - Station 06-4	170 m	EU194815	EU194797

R7	1	S62°52.843'	W58°13.816'	SEA; Falkland Islands - 17-OT20	505 m	EU194819	EU194798
S8	1	S54°49'	W60°16'	SEA; Argentinean Waters - Station 06-6	110 m	EU194818	EU194797
T8	1	S54°27.320'	W63°52.656'	BB; North of Isla de los Estudians - Station 04-7	108 m	EU194820	EU194797
U8	1	S54°27.320'	W63°52.656'	BB; North of Isla de los Estudians - Station 04-7	108 m	EU194823	EU194799
U8	1	S54°27.320'	W63°52.656'	BB; North of Isla de los Estudians - Station 04-7	108 m	EU194823	EU194797
V8	1	S53°16.214'	W66°23.150'	BB; Argentinean Waters - Station 06-1	96 m	EU194814	EU194797
W8	1	S54°27.320'	W63°52.656'	BB; North of Isla de los Estudians - Station 04-7	108 m	EU194822	EU194799
X8	1	S54°27.320'	W63°52.656'	BB; North of Isla de los Estudians - Station 04-7	108 m	EU194821	EU194797
Y8	1	S54°27.320'	W63°52.656'	BB; North of Isla de los Estudians - Station 04-7	108 m	EU194824	EU194797

Table S2: Collection information, age class, and GenBank accession numbers for nemerteans other than *P. corrugatus* from in South America, Antarctica and the sub-Antarctic Islands. Station numbers 04-xx and 06-xx were collected on the 2004 and 2006 cruises aboard the R/V Lawrence M. Gould, respectively. Others were either obtained from the ICEFISH cruises (via S.J. Lockhart).

Age class	Accession #	Latitude	Longitude	Collection Location	Depth (m)
Adults	EU718358	S 64°53.120	W 62°54.02'	South Shetlands	---
	EU718362	S 54°36.92'	W 61°07.11'	LMG04-16	200-238m
	EU718363	S 54°36.92'	W 61°07.11'	LMG04-16	200-238m
	EU718364	S 61°09.843'	W 55°51.625'	LMG04-33	117m
	EU718365	S 63°40.00'	W 57°19.75'	LMG04-40	335m
	EU718366	S 64°41.3'	W 65°55.6'	LMG04-85	368m
	EU718367	S 54°80'	W 57°01'	6-BT5	185m
	EU718368	S 54°80'	W 57°01'	6-BT5	185m
	EU718369	S 51°60'	W 57°48'	19-BT10	130m
	EU718370	S 53°47'	W 60°42'	LMG06-04	170m
	EU718371	S 53°47'	W 60°42'	LMG06-04	170m
	EU718372	S 54°20.48'	W 60°59.63'	LMG06-07	125m
	EU718373	S 54°20.48'	W 60°59.63'	LMG06-07	125m
	EU718374	S 54°20.48'	W 60°59.63'	LMG06-07	125m
	EU718375	S 62°56.004'	W 61°28.751'	LMG06-14	188m
	EU718376	S 62°18.834'	W 61°45.086'	LMG06-17	334m
	EU718377	S 62°18.834'	W 61°45.086'	LMG06-17	334m
	EU718378	S 62°18.834'	W 61°45.086'	LMG06-17	334m
	EU718379	S 62°18.834'	W 61°45.086'	LMG06-17	334m
	EU718380	S 62°18.834'	W 61°45.086'	LMG06-17	334m
	EU718381	S 62°18.834'	W 61°45.086'	LMG06-17	334m
	EU718382	S 63°08.838'	W 57°07.441'	LMG06-21	146-192m
	EU718383	S 63°08.838'	W 57°07.441'	LMG06-21	146-192m
	EU718384	S 63°08.838'	W 57°07.441'	LMG06-21	146-192m
	EU718385	S 63°08.838'	W 57°07.441'	LMG06-21	146-192m
	EU718386	S 62°34.209'	W 60°02.782'	LMG06-24	200m
	EU718387	S 66°36.663'	W 68°18.533'	LMG06-31	261m
	EU718388	S 67°47.527'	W 68°32.345'	LMG06-34	490m
	EU718389	S 68°01.172'	W 67°40.243'	LMG06-36	208m
	EU718390	S 68°01.172'	W 67°40.243'	LMG06-36	208m
	EU718391	S 68°11.161'	W 67°35.682'	LMG06-37	232m
	EU718392	S 68°11.161'	W 67°35.682'	LMG06-37	232m

	EU718393	S 68°11.161'	W 67°35.682'	LMG06-37	232m
	EU718394	S 67°39.803'	W 68°14.695'	LMG06-47	170m
	EU718395	S 65°39.843'	W 68°02.224'	LMG06-52	282m
	EU718396	S 65°01.554'	W 63°18.232'	LMG06-60	400m
	EU718397	S 65°01.554'	W 63°18.232'	LMG06-60	400m
	EU718398	S 65°01.554'	W 63°18.232'	LMG06-60	400m
	EU718399	S 65°01.554'	W 63°18.232'	LMG06-60	400m
	EU718400	S 65°01.554'	W 63°18.232'	LMG06-60	400m
Larvae	EU718401	S 62°57.72'	W 59° 2.93'	LMG04-90	200m
	EU718402	S 62°57.72'	W 59°22.93'	LMG04-90	200m
	EU718403	S 62°57.72'	W 59° 2.93'	LMG04-90	200m
	EU718404	S 63°37.00'	W 59°49.40'	LMG04-46	200m
	EU718405	S 63°37.00'	W 59°49.40'	LMG04-46	200m
	EU718406	S 63°37.00'	W 59°49.40'	LMG04-46	200m
	EU718407	S 63°37.00'	W 59°49.40'	LMG04-46	200m
	EU718408	S 62°58.23	W 60°38.76'	LMG04-127	200m
	EU718409	S 62°58.23'	W 60°38.76'	LMG04-127	200m
	EU718410	S 62°58.23'	W 60°38.76'	LMG04-127	200m
	EU718411	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718412	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718413	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718414	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718415	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718416	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718417	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718418	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718419	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718420	S 68°11.03'	W 67°37.12'	LMG06-37-PT32	200m
	EU718421	S 68°11.03'	W 67°37.12'	LMG06-37-PT32	200m
	EU718422	S 54°25.97'	W 59°31.89'	LMG04-12D	200m
	EU718423	S 68°33.93'	W 70°16.44'	LMG06-42-PT36	200m
	EU718424	S 68°36.95'	W 70°12.95'	LMG04-43-PT37	200m
	EU718425	S 68°33.93'	W 70°16.44'	LMG06-43-PT37	200m
	EU718426	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
	EU718427	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
	EU718428	S 62°57.72'	W 59°22.93'	LMG04-90	200m

EU718429	S 62°57.72'	W 59°22.93'	LMG04-90	200m
EU718431	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
EU718433	S 62°57.72'	W 59° 22.93'	LMG04-90	200m
EU718434	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
EU718435	S 62°57.72'	W 59°22.93'	LMG04-90	200m
EU718436	S 62°57.72'	W 59°22.93'	LMG04-90	200m
EU718437	S 62°57.72'	W 59° 2.93'	LMG04-90	200m
EU718438	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
EU718439	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
EU718440	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
EU718441	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
EU718442	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
EU718443	S 63°40.29'	W 56°44.93'	LMG04-42D	200m
EU718444	S 62°58.23'	W 60°38.76'	LMG04-127	200m
EU718445	S 62°57.72'	W 59° 2.93'	LMG04-90	200m
EU718446	S 62°57.72'	W 59° 2.93'	LMG04-90	200m
EU718447	S 62°57.72'	W 59° 2.93'	LMG04-90	200m
EU718448	S 62°58.23'	W 60°38.76'	LMG04-127	200m
EU718450	S 66°31.35'	W 70°00.03'	LMG04-14-PT50	200m
EU718451	S 64°23.54'	W 62°59.82'	LMG06-10-78	200m
EU718452	S 65°50.51'	W 66°59.83'	LMG06-30-PT26	200m
EU718453	S 68°33.93S	W 70°16.44'	LMG06-42-PT36	200m
EU718454	S 68°33.93S	W 70°16.44'	LMG06-42-PT36	200m
EU718455	S 68°33.93S	W 70°16.44'	LMG06-42-PT36	200m
EU718456	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718457	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718458	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718459	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718460	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718461	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718462	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718463	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718464	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718465	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718466	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718467	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m

EU718468	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m
EU718469	S 65°02.41'	W 65°59.92'	LMG04-14-PT46	200m

Table S3 Genetic distance statistics for *Parborlasia corrugatus* mtDNA genes (16S, COI, and the third positions of COI).

mtDNA Gene	nh	π	h	p	No. Variable Sites	No. Parsimony Informative Sites	% G-C Content
16S	9	0.00827	0.722	0.01782	13	9	31.8%
COI	25	0.04877	0.972	0.10184	81	68	40.7%
COI third position	23	0.13432	0.964	0.27500	72	61	43.2%

nh , number of haplotypes detected; π , nucleotide diversity; h , haplotype diversity; p , maximum uncorrected genetic distance.

Supplemental Figure Legends

Fig. S1: Examples of the morphological variation encountered in *Parborlasia* samples from the Antarctic Peninsula (a through d; haplotypes L1, A1, D1, and A2 respectively), Burdwood Bank (e; haplotype U8), and Southeastern Argentina (f; haplotype O8). All of the specimens depicted here were included in the molecular analysis. Note the plasticity in pigmentation and the anterior banding pattern (i.e., the light colored “collar”) between specimens. No obvious morphological differences were identified that corresponded with geography or the genetic differences reported in this study. Images are not to scale relative to one another.

Figure S1:

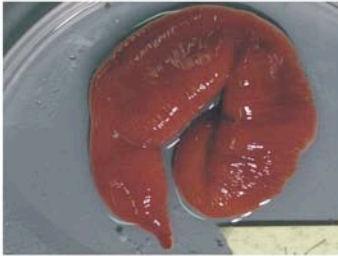
(a)



(b)



(c)



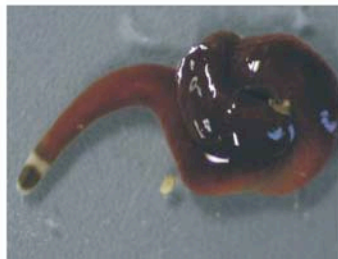
(d)



(e)



(f)



Supplemental Text:

Results:

Morphology of whole and sectioned specimens of *P. fueguina* (USNM 081538 – 081557), *P. landrumae* (USNM 081595 – 081597) and *P. corrugatus* (USNM 069919 – 069948, 086472 – 086597) already in the Smithsonian collections were examined to assess the reliability of morphological features to distinguish these described species. No cephalic pigment pattern was present in the available specimens of any of these species (Gibson 1983, 1985). In the case of *P. corrugatus* we have ample evidence that pigment and patterning is lost in alcoholic preservative (Norenburg, pers. obs.). The epidermal ground colour of *P. corrugatus* generally was uniform and included almost white, yellow, brown, reddish brown, gray and dark gray or dark brown. *Parborlasia fueguina* included whitish and dark brown individuals. All *P. landrumae* were whitish. External morphology of the latter two species fell within the range of variation exhibited by *P. corrugatus* also identified by Gibson (1983).

Gibson (1985) assigned worms to *P. fueguina* on the basis of two internal diagnostic characters: presence of a large anterior rhynchocoelic diverticulum compressing the foregut, and a conical valve connecting the pylorus to the intestine. The former was present in several of the section series but is interpreted here as having a high probability of being a ballooning artefact that often occurs at fixation, even in well-relaxed specimens. The second diagnostic is evident in only one section series (USNM 081539) and is a clear misinterpretation; there is in fact a wide transition, as is typical of other heteronemerteans.

The following internal anatomical features are alleged to distinguish *P. landrumae* from other *Parborlasia*: an incomplete stratum of proboscis outer longitudinal muscle (OLM), a massive post-cerebral blood lacuna (PBL), remnants of a longitudinal muscle plate (LMP) to either side of the mid-dorsal blood vessel (DBV), two villar projections along the rhynchocoelic portion of the DBV, high degree of cephalic gland development, and smaller number of eyes (Gibson 1985). The two available sectioned specimens of *P. landrumae* were both sexually immature. The incomplete nature of the proboscis OLM was verified for one specimen, but proboscis structure varies regionally and by size in most heteronemerteans and warrants detailed comparative study for multiple specimens (Norenburg pers. obs.). A massive post-cerebral blood vessel and remnants of LMP along the DBV also were observed in several individual *P. corrugatus*. The two villar projections appeared to be an over-interpreted fixation artefact and were evident in only one of the two specimens. Cephalic gland development did not seem unusual with respect to similarly sized *P. corrugatus*. Numbers of ocelli were not compared.

Discussion:

Many species of nemerteans have been named and described as new based on internal anatomical diagnostics inferred from histological study of specimens lacking useful external diagnostics. Oftentimes this occurred because specimens were not annotated in life and were poorly preserved in the field without benefit of relaxation. Internal anatomy of almost all such specimens is greatly compromised by powerful contraction, particularly of the body-wall muscles but also the many other muscle systems (Norenburg pers. obs.), as is the case for all the museum specimens examined here. Discovering unambiguous internal diagnostics to distinguish closely related species often fails even in relaxed specimens, (e.g., Strand *et al.* 2005, Schwartz & Norenburg 2005). Thus, it appears that nemertean

morphology may be relatively conserved with respect to genetic differentiation. However, genetic studies have demonstrated instances both of significant intraspecific plasticity in pigment patterning (e.g., Sundberg & Andersson 1995) and of external morphological crypsis among distantly related species of nemerteans (e.g., Rogers *et al.* 1995). *Parborlasia corrugatus* is somewhat unusual among nemerteans in encompassing specimens with a wide range of ground-colour variation (Wheeler 1934, Gibson 1983). Epidermal ground colour may be influenced by source of food (Norenburg pers. obs.). *Parborlasia corrugatus* specimens from the type locale were reported to have white markings lining the anterior two-thirds of the cephalic slits, often with posterior vertical extensions that may meet to form a transverse white bar across the dorsum of the head. Presence of both the white cephalic margins and of the dorsal transverse bar are alleged to be variable (Wheeler 1934). White margins were present in all specimens sampled for DNA in the present study, but the white transverse bar was not always complete.

Gibson (1985) apparently distinguished specimens of putative *P. fueguina* and *P. landrumae* as different from the hundreds of specimens otherwise assumed to be *P. corrugatus* after observing presumed diagnostic differences in histological sections. The remaining whole specimens allocated by him to the first two species have in common primarily that they are very small and have a relatively small mouth. The latter can be a diagnostic feature, but is far from unambiguous and can be greatly influenced by size of specimen, by individual and general response to various fixation protocols and by initial conditions (e.g., vitality of specimens).

In life, *P. fueguina* is recognized by a yellow transverse cephalic band, which was not observable by Gibson (1985) in any of his specimens. Gibson (1985) stated that several of his specimens had evidence of a post-cephalic ligature, which was given diagnostic significance by Serna de Esteban & Moretto (1968). However, this is likely a common fixation artefact for heteronemerteans (Norenburg pers. obs.).

All specimens studied for morphology showed evidence of extreme contraction, which greatly enhanced the prospect of inducing fixation artefacts, some of which are likely to occur commonly if not consistently – consistency is where the problem lies. Gibson (1983, 1985) acknowledges these kinds of problems in discussing the numerous synonymies for *P. corrugatus*. We conclude that there is little likelihood of recognizing morphological diagnostics to distinguish the putative cryptic species of *P. corrugatus* suggested by our genetic data, especially as they were all preserved with minimal annotation in life and no relaxant.

The inclusion of *P. fueguina* is relevant here because specimens matching the original description have never been reported from Antarctic waters, whereas it is supposed to be relatively common in Tierra del Fuego. Gibson (1985) assigned to this species 27 specimens from localities south of the ACC, none of which had any pigment patterning. Hence, we could reasonably have expected it to occur in our Argentinean samples but did not encounter it there or among our Antarctic samples, which begs the question why. We did not find morphologically identifiable living *P. fueguina* in our Argentinean samples, perhaps because they have a restricted distribution. However, Gibson's (1985) specimens were mostly trawled from similar habitats as sampled by us. One interpretation consistent with the genetic data and our morphological re-evaluation is that the specimens assigned to *P. fueguina* by Gibson (1985) belong to the Antarctic *P. corrugatus*.

Although *P. landrumae* was described from outside the range of this study, it is inside the alleged distribution range of *P. corrugatus* and is relevant here because our morphological examination raises significant doubt that the above section series are adequate to reliably distinguish the worms from *P. corrugatus*, especially small specimens. The nine specimens assigned to *P. landrumae* are from a single dredge haul. Thus if *P. landrumae* represents a true biological species, we propose that a *Parborlasia*-like species with a genetic profile significantly different from Antarctic *P. corrugatus* should be relatively common at the type locale of *P. landrumae*.

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