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).

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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).

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Larvae

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

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<th>π</th>
<th>h</th>
<th>p</th>
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<td>9</td>
<td>0.00827</td>
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<td>0.01782</td>
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<td>0.10184</td>
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*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 encounter 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:
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 nemertans (e.g., Rogers et al. 1995).

*Parborlasia corrugatus* is somewhat unusual among nemertans 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 heteronemertans (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*.

References:


