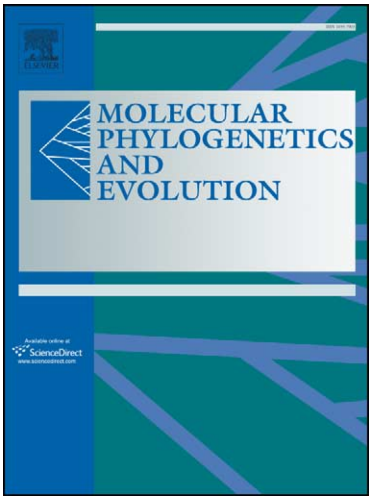

Molecular insights indicate that *Pachycara thermophilum* (Geistdoerfer, 1994) and *P. saldanhai*(Biscoito and Almeida, 2004) (Perciformes: Zoarcidae) from the Mid-Atlantic Ridge are synonyms species

S. Stefanni, F.M. Porteiro, R. Bettencourt, P.J. Gavaia, R.S. Santos

PII:	S1055-7903(07)00019-X
DOI:	10.1016/j.ympev.2007.02.001
Reference:	YMPEV 2471
To appear in:	*Molecular Phylogenetics and Evolution*
Received Date:	5 January 2007
Revised Date:	31 January 2007
Accepted Date:	1 February 2007

Please cite this article as: Stefanni, S., Porteiro, F.M., Bettencourt, R., Gavaia, P.J., Santos, R.S., Molecular insights indicate that *Pachycara thermophilum* (Geistdoerfer, 1994) and *P. saldanhai*(Biscoito and Almeida, 2004) (Perciformes: Zoarcidae) from the Mid-Atlantic Ridge are synonyms species, *Molecular Phylogenetics and Evolution* (2007), doi: 10.1016/j.ympev.2007.02.001

Short Communication

Molecular insights indicate that *Pachycara thermophilum* (Geistdoerfer, 1994) and *P. saldanhai* (Biscoito and Almeida, 2004) (Perciformes: Zoarcidae) from the Mid-Atlantic Ridge are synonyms species

Stefanni S.[1], Porteiro F.M.[1], Bettencourt R.[1], Gavaia P.J.[2] and Santos R.S.[1]

[1] *IMAR, Dept of Oceanography and Fisheries, University of the Azores – 9901-862, Horta, Azores, Portugal*

[2] *CCMAR, University of the Algarve, Campus de Gambelas - 8005-139, Faro, Portugal*

Introduction

The genus *Pachycara* comprises of 20 species distributed across the globe. Due to difficulties in collecting these fish, most of the species were described from single or few individuals. Until now, the systematics for these species has been based on traditional taxonomy (Froese and Pauly, 2006) and the only studies with molecular interest were performed on the Circum Antarctic *P. brachycephalum* (Ritchie et al., 1996; Lucassen et al., 2003; Cheng et al., 2006; Mark et al., 2006).

Seven species of *Pachycara* are known in the Atlantic Ocean of which, *P. thermophilum* (Geistdoerfer, 1994) and *P. saldanhai* (Biscoito and Almeida, 2004) are considered endemic of the hydrothermal vents fields from the Mid Atlantic Ridge (MAR).

P. thermophilum has been recorded from several vent fields, such as Logatchev at 3000 m, Snake Pit at 3480 m, Trans-Atlantic Geotraverse (TAG) at 3600 m and Broken Spur at 3020 m (Geistdoerfer, 1994, 1997; Parin, 1995; Anderson and Bluhm, 1996). *P. saldanhai*, the most recently described species (Biscoito and Almeida, 2004), was only recorded from the Rainbow hydrothermal vent field at 2280 m (Fig. 1). The taxonomic differences that lead to the description of *P. saldanhai* as a new species are not completely obvious; in fact, this species shares with *P. thermophilum* several characters. The two major differences defining *P. saldanhai* are based on the lateral line that has two branches and slightly higher number of caudal vertebrae (Biscoito and Almeida, 2004).

In this study, we use molecular tools to establish the level of genetic variation among mtDNA sequences of *P. thermophilum* and *P. saldanhai*. For this purpose, the mtDNA regions amplified for the comparison are fragments including the complete sequence of the highly variable control region (CR) and a partial sequence of the cytochrome oxidase subunit I (COI).

Material and Methods

Samples

All *P. thermophilum* specimens were caught in 2001 during the DIVERSExpedition on the Mid-Atlantic Ridge using the DSV *Alvin* on board the R/V *Atlantis*. Two individuals (3666-1 and 3667-1) were collected during 6-7 July 2001 from the Logatchev vent field (Irina 2 site: 14º45.2'N; 044º58.8'W) at 3007 m depth. The other nine specimens (3672-1/2/3, 3673-1/2/3/4/5 and 3674-1) were collected during 14-16 of July 2001 from the Snake Pit vent field (Moose site: 23º22.9'N; 044º55.8'W) at 3490-3492 m depth.

The two specimens of *P. saldanhai* (Psal01 and Psal02) were captured in 2006 from the Rainbow vent field (EXO2 site: 36º13.8'N; 033º54.2'W) at 2309 m depth These were collected during the MOMARETO cruise, using the ROV *Victor 6000* on board of the R/V *Pourquoi pas?*.

Individuals were collected with the slurp-gun of the submersibles employed during specific cruises with the exception of *P. thermophilum* 3674-1, which was caught in a fish trap.

Molecular analyses

From all specimens of *Pachycara*, a small portion of muscle tissue was preserved in 80% ethanol before being stored at -80ºC. DNA extracts were prepared following the phenol/chloroform protocol (Sambrook et al., 1989) with slight modifications.

Fragments of the mtDNA including the complete sequence for the control region (CR) were amplified using the following primer sequences: *Pro* (Nesbo et al., 2000) and *12S* (Nesbo et al., 1998). Partial cytochrome oxidase subunit I (COI) was amplified using *FishF1* and *FishR1* primers (Ward et al., 2005). Specifically designed oligos were used for sequencing the CR, while the same pairs used for PCR amplification were also used for sequencing the COI region.

The thermal cycling profile started with 94ºC for 2 min. followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 53ºC (for CR) or 54ºC (for COI), extension at 72ºC for 1 min. 30 s (for CR) or 1 min. (for COI), with a final extension at 72ºC for 10 min.

All sequences (GenBank accession Nos. EF1405991 - EF140616) were aligned using SEAVIEW (Galtier et al., 1996) and boundaries of the mtDNA regions were defined from comparison with sequences already deposited in GenBank using BLAST programme (Altschul et al., 1990).

Levels of genetic diversity were estimated using the program ARLEQUIN 2.000 (Schneider et al., 2000). Haplotypic diversity (h) and nucleotide diversity (π), including their standard deviations, were calculated for each of the mtDNA regions.

Results

All specimens were successfully sequenced for both mtDNA regions.

The complete sequence of the CR defined 4 distinct haplotypes among the 13 sequences. The shortest haplotype was 865bp long and it was shared with 4 specimens of *P. thermophilum* (3672-3, 3673-1/3 and 3674-1) and the 2 individuals of *P. saldanhai* (Psal01 and Psal02). The other three haplotypes were 866bp long, two of which represented by single specimens (*P. thermophilum* 3673-2 and 3673-5), while the last haplotype was shared with 5 specimens of *P. thermophilum* (3666-1, 36667-1, 3672-1/2, 3673-4). For complete alignment one indel in position 316 was required.

The aligned sequences of CR of *Pachycara* contained a total of 8 polymorphic sites, giving an overall haplotypic diversity (h) of 0.6795±0.0885, nucleotide diversity (π) of 0.0028±0.0018 and the nucleotide composition was estimated to be A=0.3070, C=0.2119, T=0.3052 and G=0.1760.

Sequences from the partial COI had a length of 659bp and no indels were necessary for alignment. From comparison with homologous sequences deposited in GenBank, these fragments start from position 51. The 13 sequences defined 5 haplotypes, 3 of which were represented by single specimens (*P. thermophilum* 3673-2 and 3673-5 and *P. saldanhai* Psal01). The other 2 haplotypes were shared with other fish. In one there are 7 specimens of *P. thermophilum* (3666-1, 3667-1, 3672-1/2/3, 3673-2/4) and in the other there are 2 specimens of *P. thermophilum* (3673-1, 3674-1) and 1 individual of *P. saldanhai* (Psal02).

The aligned sequences of COI of *Pachycara* contained a total of 9 polymorphic sites, giving an overall haplotypic diversity (h) of 0.6923±0.1187, nucleotide diversity (π) of 0.0035±0.0023 and the nucleotide composition was estimated to be A=0.2103, C=0.3157, T=0.2706 and G=0.2033.

Discussion

This study has not been able to differentiate the two species of *Pachycara* living in MAR hydrothermal vent fields (*P. thermophilum vs. P. saldanhai*) from mtDNA molecular sequences. The highly polymorphic CR, which is particularly suited for intraspecific studies due to the substantial mutation rate (e.g. Brown et al., 1979; Stefanni and Thorley, 2003; Stefanni and Knutsen, 2007), defined an identical haplotype for the 2 specimens of *P. saldanhai* and 4 of *P. thermophilum*.

A similar result was obtained from the comparison of the partial COI region, the portion of the mtDNA considered very efficient for discriminating between species (Hebert et al., 2004a,b; Hogg and Hebert, 2004) and therefore adopted for a DNA-based taxonomic system as the “Barcoding of Life” (Hebert et al., 2003). Efficacy of this fragment for identifying fish species is supported by several studies (e.g. Ward et al., 2005). However, when the two putative species of *Pachycara* where compared, COI did not discriminate among them. Although separate haplotypes were defined from the two specimens of *P. saldanhai*, one of the sequences was shared with 2 individuals of *P. thermophilum*.

The molecular data indicate a lack of differentiation among the specimens of *Pachycara* species collected from Logatchev, Snake Pit and Rainbow vent fields, suggesting that *P. saldanhai* is a synonymous species with *P. thermophilum*.

Nevertheless, if this species of *Pachychara* is indeed strictly associated to hydrothermal vent fields, as suggested by Biscoito et al. (2002), our results are interesting when compared to the findings presented for the hydrothermal mussels from the same vent fields. In fact, the northern and southern vent fields of the MAR are inhabited by distinct species, *Bathymodiolus azoricus* and *B. puteoserpentis* (Maas et al., 1999; Von Cosel et al., 1999; Comtet et al., 2000; Jones et al., 2006), with a hybrid zone at Broken Spur in which both species are present and hybridize (Won et al., 2003).

Logatchev is located at about 1400 nm distance from the Rainbow site with three other vent fields in between. Considering the hypothesis that *P. thermophilum* is endemic for the vents, we could expect that there are several more unknown hydrothermal vent fields functioning as stepping-stones between those presently discovered as we are dealing with large distances.

An alternative hypothesis is to consider this species as widespread bathybenthic, being regularly distributed along the MAR, but also occurring and exploiting the biomass at hydrothermal vent fields. To date, there are no other records for this species occurring outside the hydrothermal vent fields. This is not surprising given that: 1) the *P. thermophilum* is a highly cryptic species; 2) non-vent sites at the ridge are poorly studied when compared with vent sites, probably the best studied and visually scrutinized and sampled ecosystems in the deep-sea.

Better knowledge of physiological adaptations to cope with the specific conditions at the vents and of the reproductive strategies developed by *Pachycara* combined with more specific studies on genetic structure of the demographic units living inside each field would help to clarify how gene flow between the demographic units from different vent fields is maintained.

To conclude, we suggest a revision of the nomenclature considering *P. thermophilum* (Geistdoerfer, 1994) as the only *Pachycara* species inhabiting the North Atlantic vent fields, so far described to science. Consequently, the morphological differences reported by Biscoito and Almeida (2004) may eventually be considered phenotypic expressions associated to the large geographical distance that separates these vents.

Acknowledgements

We are extremely thankful for the opportunities offered for sample collection provided by the DIVERSExpedition in 2001 (CL Van Dover as Chief Scientist and project funded by the National Science Foundation, Biological Oceanography) and MOMARETO cruise (J Sarrazin and P-M Sarradin as Chief scientists and project funded by the FP6 European Commission Exocet/D project: GOCE-CT-2003-505342). This work was also partially funded by the grant POCTI/CVT/42098/2001 (FishDev) from FCT (Foundation for Science and Technology, Portugal). S.S., R.B. and P.J.G. are postdoctoral fellows funded by FCT, refs SFRH/BPD/14981/2004, SFRH/BDP/14896/2004 and SFRH/BPD/14528/2003, respectively. We would like to thank G. Carreira (IMAR/DOP) and M. Segonzac (IFREMER) as they were involved in sampling during the DIVERSExpedition in 2001. We are very grateful to captains and crews of the research vessels involved in the projects, as well as the pilots of the ROVs. This paper is also a contribution to MarBEF (Network of Excellence: “Marine Biodiversity and Ecosystem Functioning” – contract nr. GOCE-CT-2003-505446). IMAR/DOP is funded through the pluri-annual and programmatic funding schemes of FCT (Portugal) and DRCT (Azores, Portugal) as Research Unit #531 and Associate Laboratory #9.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403-410.

Anderson, M.E., Bluhm, H., 1996. Description of new species of *Pachycara* Zugmayer, 1911 from the abyssal south-eastern Pacific and redescription of *P. thermpphilum* Geistdoerfer, 1994, with a new key to the species**.** *Transactions of the Royal Society of South Africa* **51**, 219-227.

Biscoito, M., Almeida, A.J., 2004. New species of *Pachycara* Zugmayer (Pisces: Zoarcidae) from the Rainbow hydrothermal vent field (Mid-Atlantic Ridge). *Copeia* **3**, 562-568.

Biscoito, M., Segonzac, M., Almeida, A.J., Sebruyères, D., Geistdoerfer, P., Turnipseed, M, and Van Dover, C., 2002. Fishes from the hydrothermal vents and cold seeps – An update. *Cahiers de Biologie Marine* **43**, 359-362

Brown, W.N., George, M., Wilson, A.C., 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences, USA* **76**, 1967-1971.

Cheng, C.-H.C., Cziko,P.A., Evans,C.W., 2006. Nonhepatic origin of notothenioid antifreeze reveals pancreatic synthesis as common mechanism in polar fish freezing avoidance. *Proceedings of the National Academy of Sciences, USA* **103**, 10491-10496.

Comtet, T., Jollivet, D., Dixon, D.R., 2000. Molecular and morphological identification of settlement-stage vent mussel larvae, *Bathymodiolus azoricus* (Bivalvia: Mytilidae), preserved in situ at active vent fields on the Mid-Atlantic Ridge. *Limnology and Oceanography* **45**: 1655-1661.

Froese, R., Pauly, D., 2006. FishBase. World Wide Web electronic publication. www.fishbase.org, version (09/2006).

Geistdoerfer, P., 1994. *Pachycara thermophilum*, une nouvelle espèce de poisson Zoarcidae des sites hydrothermaux de la dorsale médio-Atlantique. *Cybium* **18**, 109-115.

Geistdoerfer, P., 1997. *Pachycara thermophilum* Geistdoerfer 1994, p 226. *In:* Handbook of deep-sea hydrothermal vent fauna. D. Desbruyères and M. Segonzac (eds.). Éditions IFREMER, Brest, France.

Hebert, P.D.N., Cywinska, A., Ball, S.L., de Waard, J.R., 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London, Series B: Biological Sciences (London)* **270**, 313-322.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., Hallwachs, W., 2004a. The species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences, USA* **101**, 14812-14817.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S., Francis, C.M., 2004b. Identification of birds through DNA barcodes. *Public Library of Science, Biology* **2**, 1657-1663.

Hogg, I.D., Hebert, P.D.N., 2004. Biological identification of springtails (Collembola: Hexapoda) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology* **82**, 749-754.

Jones, W.J., Won, Y-J., Maas, P.A.Y., Smith, P.J., Lutz, R.A., Vrijenhoek, R.C., 2006. Evolution of habitat use by deep-sea mussels. *Marine Biology* **148**: 841-851.

Lucassen, M., Schmidt, A., Eckerle, L.G., Portner,H.O., 2003. Mitochondrial proliferation in the permanent vs. temporary cold: enzyme activities and mRNA levels in Antarctic and temperate zoarcid fish. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **285**, 1410-1420.

Mark,F.C., Lucassen,M., Poertner,H.O., 2006. Thermal sensitivity of uncoupling protein expression in polar and temperate fish. *Comparative Biochemistry and Physiology, Part D Genomics Proteomics* 1, 365-374.

Nesbo, C.L., Arab, M.O., Jakobsen, K.S., 1998. Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid species (*Perca fluviatilis, Acerina cernua, Stizustedion lucioperca*). *Genetics* **148**, 1907-1919.

Nesbo, C.L., Rueness, E.K., Iversen, S.A., Skagen, D.W., Jakobsen, K.S., 2000. Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a genealogical approach reveals genetic structuring among the eastern Atlantic stocks. *Proceedings of the Royal Society of London, Series B: Biological Sciences (London)* **267**, 281-292.

Parin, N.V., 1995. Deep-Sea fishes. *Haptenchelys texis* (Synaphobranchidae) and *Pachycara thermophilum* (Zoarcidae) from Broken Spur Hydrothermal Vent Field (Mid-Atlantic Ridge). *Journal of Ichthyology* **35**, 328-332.

Ritchie, P.A., Bargelloni, L., Meyer, A., Taylor, J.A., Macdonald, J.A., Lambert, D.M.,1996. Mitochondrial phylogeny of trematomid fishes (Nototheniidae, Perciformes) and the evolution of Antarctic fish. *Molecular Phylogenetics and Evolution* **5**, 383-390.

Sambrook, J., Fritsch, E.F., Manitais, T., 1989. Molecular Cloning: a Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Schneider, S., Roessli, D., Excoffier, L., 2000. *ARLEQUIN, version 2.000: A software for population genetics data analysis.* Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Stefanni, S., Knutsen, H., 2007. Phylogeography and demographic history of the deep-sea fish, *Aphanopus carbo* (Lowe, 1839), in the NE Atlantic: vicariance followed by secondary contact or speciation? *Molecular Phylogenetics and Evolution* **42**: 38-46.

Stefanni, S., Thorley, J.L., 2003. Mitochondrial DNA phylogeography reveals the existence of an Evolutionary Significant Unit of the sand goby *Pomatoschistus minutus* in the Adriatic (Eastern Mediterranean). *Molecular Phylogenetics and Evolution* **28**, 601-609.

Von Cosel, R., Comtet, T., Krylova, E., 1999. *Bathymodiolus* (Bivalvia:Mytilidae) from hydrothermal vents on the Azores triple junction and the Logatchev hydrothermal field, Mid-Atlantic Ridge. *The Veliger* **42**:218–248

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences (London)* **360**, 1847-1857.

Won, Y, Hallam, S.J., O'Mullan, G.D., Vrijenhoek, R.C., 2003. Cytonuclear disequilibrium in a hybrid zone involving deep-sea hydrothermal vent mussels of the genus Bathymodiolus. *Molecular Ecology* **12**:3185-90.

Legends:

Fig. 1. Map indicating the positions and depths of the hydrothermal vent fields on the Mid-Atlantic Ridge.

Figures:

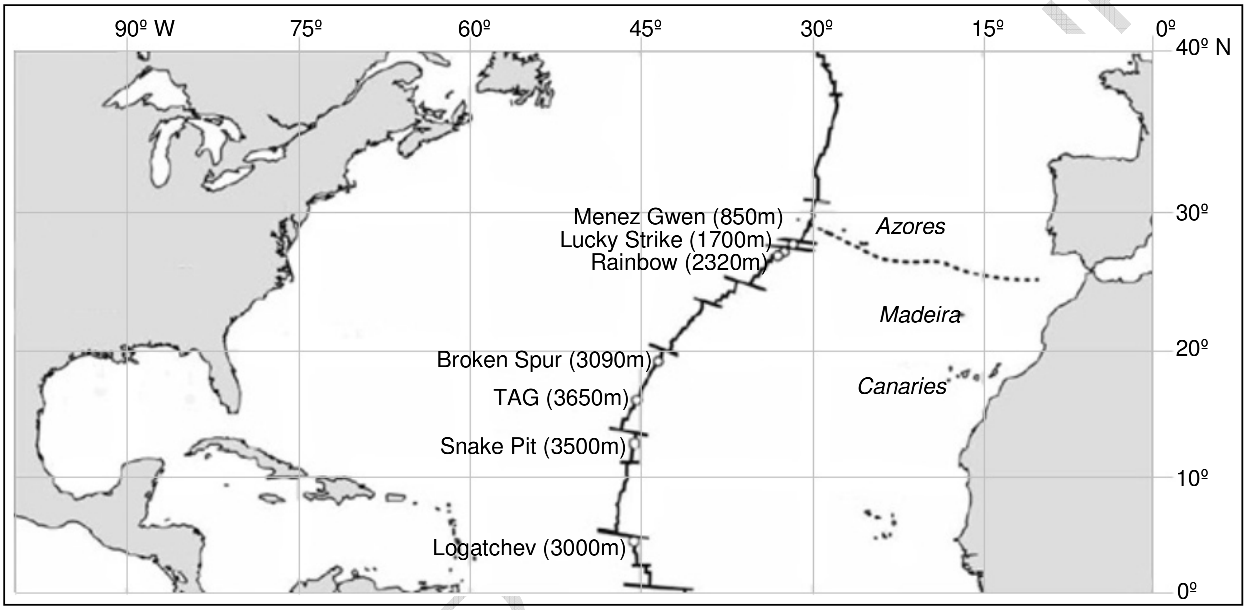

Figure 1