The ‘scaly-foot gastropod’: a new genus and species of hydrothermal vent-endemic gastropod (Neomphalina: Peltospiridae) from the Indian Ocean

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ABSTRACT

The ‘scaly-foot gastropod’ is widely recognized as an iconic species of deep-sea hydrothermal vent ecosystems in the Indian Ocean. Uniquely among gastropods, this species carries hundreds of dermal sclerites on its foot and these scales can be covered in iron sulphide that also covers its shell, making it the only extant metazoan known to utilize iron sulphide as part of its skeleton. It has not been formally named, despite attracting great attention from both scientists and the general public alike, although a manuscript name has occasionally been used in various sources. The RRS James Cook JC67 expedition in 2011 sampled the biota of the Longqi vent field (37°47.027’S, 49°38.963’E), Southwest Indian Ridge, for the first time, revealing a previously unknown population of the ‘scaly-foot gastropod’. The present study gives a formal name to the ‘scaly-foot gastropod’, Chrysomallon squamiferum n. gen., n. sp. with Longqi vent field as the type locality. The erection of the new monotypic genus is supported by both morphological and molecular characterization, differentiating it from existing genera of the family Peltospiridae. Analysis of the cytochrome c oxidase subunit I gene reveals a 24–26% pairwise distance between Chrysomallon and five other genera of Peltospiridae, while the range among those five genera is 14–25%. The new genus is placed in the family Peltospiridae based on morphological characteristics, including lack of sexual dimorphism, no copulatory organ, the distal end of marginal teeth being subdivided into many denticles and the ventral margin of the gill leaflets carrying a series of bulges. A five-gene Bayesian phylogenetic reconstruction does not contradict the placement within Peltospiridae.

INTRODUCTION

The first hydrothermal vent field located in the Indian Ocean, the Kairei vent field (25°19.23’S, 70°02.42’E; Fig. 1) on the Central Indian Ridge (CIR), was discovered by the RV Kairei KR00-05 cruise in 2000 (Hashimoto et al., 2001) and subsequently surveyed by the RV Knorr 162-15 expedition in 2001 (Van Dover et al., 2001). This survey yielded one of the most peculiar deep-sea hydrothermal-vent gastropods known to date, referred to as the ‘scaly-foot gastropod’ (Warén et al., 2003). This species attracted much attention because it had hundreds of black metallic sclerites covering its foot. The soft tissue core of each sclerite was covered in conchiolin, which was in turn covered with pyrite (FeS₂) and greigite (Fe₃S₄), two forms of iron sulphide (Warén et al., 2003). Its shell was also covered in the same material, making it the only known metazoan to use iron in its skeleton (Yao et al., 2010). Recently, Nakamura et al. (2012) reported a white variety of the scaly-foot gastropod, which lacks the iron sulphide layer, from the newly discovered CIR Solitaire vent field (19°33.41’S, 65°50.89’E; Fig. 1), with genetic analyses revealing that this variety is the same species as the Kairei scaly-foot gastropod.

An extensive description of this species with anatomical details and preliminary molecular phylogenetic results based on 16S rRNA was published by Warén et al. (2003), placing it in the neomphaline family Peltospiridae; this work is sufficient for the recognition of the species and to distinguish it from all other known gastropods. Unfortunately this did not meet the requirement of the International Code of Zoological Nomenclature (ICZN, 1999), as no name was given and no type specimen was designated. There was, however, a manuscript name, Chrysomallon squamiferum, which was released with the 16S sequence data filed on GenBank; despite this being a nomen nudum it has been widely quoted in published literature (e.g. Yao et al., 2010; Nakagawa et al., 2014).

In November 2011 the RRS James Cook expedition JC67 sampled the first visually-confirmed deep-sea hydrothermal vent field on the Southwest Indian Ridge (SWIR), the Longqi vent field (37°47.027’S, 49°38.963’E; Fig. 1; Copley, 2011; Tao et al., 2012, 2014), using the remotely operated vehicle (ROV) Kiel 6000 (Copley, 2011). This survey yielded a third population
avoid further confusion in literature, we use their manuscript name for the formal description. The status of the species within the clade Neomphalina is investigated using the specimens collected from Longqi vent field (including holotype and paratypes) and also taking into account published information from the other two known populations.

MATERIAL AND METHODS

Material

The Longqi vent field (37°47.027'S, 49°38.963'E, c. 2,780 m deep; Fig. 1; Tao et al., 2014) was detected and investigated by the RV Da Yang Yi Hao expeditions DY115-19 and DY115-20 from 2007 to 2009 (Tao et al., 2012), becoming the first visually-confirmed hydrothermal vent field on the SWIR. This site was first sampled by ROV during the RRS James Cook expedition JC67 in November 2011 (Copley, 2011). Specimens of the scaly-foot gastropod were collected from the ‘Tiamat’ sulphide edifice (Copley, 2011) site using the suction sampler of the Kiel 6000 ROV and fixed in 4% buffered formalin for morphological examination and in 99% ethanol for genetic studies.

Morphology

Investigation of external morphology was carried out under a Leica ×10 dissection microscope. The radula was dissected from three specimens preserved in 99% ethanol and treated with 10% KOH solution overnight to dissolve tissue. The area containing the protoconch was excavated in adult specimens in attempts to observe the protoconch. Specimens for SEM underwent a hydration series in 75-60-40-20-0% ethanol solution and were rinsed in distilled water. The specimens underwent sonication and then a reverse dehydration series.

Genetics

Partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene were used to assess the genetic homogeneity of the Longqi, Kairei and Solitaire populations, and for comparison with other peltospirid species represented in GenBank (Table 2). For the COI analysis, five haplotypes from each of the Kairei, Solitaire and Longqi vent fields were randomly chosen. A recent five-gene phylogenetic reconstruction by Aktipis & Giribet (2012) placed Cocculinoidea as the sister clade to Neomphalina, and thus Cocculina messangi was used as the outgroup in the COI tree. Furthermore, to investigate the relatedness of the new peltospirid species to other known species of Neomphalina, sequences of five genes from six other neomphaline gastropods were used for a phylogenetic reconstruction (Table 3). The genes chosen were COI, histone 3 (H3), 16S rRNA, 18S rRNA and 28S rRNA. Non-neomphaline gastropod outgroups were selected from Vetigastropoda, Cocculinoidea, Caenogastropoda and Patelloida. Selection of taxa was limited to those with all five genes available in GenBank. Sequences used in the present study were all obtained from GenBank except for the Longqi scaly-foot gastropod, which was newly sequenced.

Genomic DNA was extracted using QIAGEN (Crawley, UK) DNeasy Blood and Tissue Kit following the manufacturer’s instructions. Primer pairs used are listed in Table 1.
The polymerase chain reaction (PCR) was carried out in 12 μl reactions, including 2 μl DNA template (100-200 ng/μl), 8 μl QIAGEN Master Mix, 0.4 μl double-distilled water, 1.6 μl primer mix containing 0.8 μl each of forward and reverse primers (4 pmol/μl). PCR was performed with the following protocol: 95 °C, 15 min, followed by 40 cycles of [94 °C, 45 s; primer-specific annealing temperature, 60 s; 72 °C, 60 s], ending with 72 °C, 5 min. The annealing temperatures used for 16S, 18S and 28S were 47 °C, for COI 45 °C and for H3 43 °C. Amplification of the desired region was confirmed with 1% agarose gel electrophoresis. Successful PCR products were purified using QIAGEN QIAquick PCR purification kit (Crawley, UK) using standard protocols. Cycle sequencing reactions were carried out with the protocol: 96 °C, 1 min, followed by 25 cycles of [96 °C, 10 s; 50 °C, 5 s; 60 °C, 4 min], ending with 60 °C, 4 min. Sequenced products were precipitated with an EDTA/ethanol method (Zengerin & Hartley, 1985). Sequences were resolved from precipitated products using an Applied Biosystems 3100 DNA sequencer (Department of Zoology, University of Oxford).

Alignment and editing of genetic sequences were carried out in Geneious v. 5.6 (Drummond et al., 2011) and reads were manually quality-checked and corrected by eye. Only good-quality sequences matching forward and reverse readings were used in downstream analyses. Pairwise distances of COI were calculated with software MEGA v. 5.05 (Tamura et al., 2007) and removed from analyses. The most suitable evolutionary model was tested using program PartitionFinder v. 1.0.1 (Lanfear et al., 2012), using scores for the Akaike information criterion. The models selected were as follows: H3, COI (first and second codons), 16S, 28S = K80+I+G; COI (third codon) = HKY+I+G; 18S = K80+G. The total sequence lengths used were 2,753 bp for the five-gene tree and 457 bp for the COI tree.

Phylogenetic reconstruction was carried out with Bayesian inference using MrBayes v. 3.2 (Ronquist et al., 2012). In both COI and five-gene analyses, Metropolis-coupled Monte Carlo Markov Chains were run for five million generations. Convergence topology samples were collected every 100 generations and the first 25% were discarded as burn-in to ensure that chains sampled a stationary position. The software Tracer v. 1.6 (Rambaut et al., 2013) was used to check for convergence and to calculate adequate burn-in values.

Table 2 shows GenBank accession numbers of sequences used in the COI tree and Table 3 shows the same for the five-gene phylogeny. New sequences generated from this study are deposited in GenBank under accession numbers KP898439-KP898447.

### Table 1. List of PCR primers used in obtaining sequences of *Chrysomallon squamiferum* n. sp. from the Longqi population specimens.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction</th>
<th>Name</th>
<th>Sequence 5'-3'</th>
<th>Citation</th>
</tr>
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<tr>
<td>H3</td>
<td>Forward</td>
<td>H3aF</td>
<td>ATG GCT CGT ACC AAG CAG ACV GC</td>
<td>Colgan et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>H3aR</td>
<td>ATAT CTC TTR GGC ATR ATR GTG AC</td>
<td></td>
</tr>
<tr>
<td>COI</td>
<td>Forward</td>
<td>LCO1490</td>
<td>GGT CAA CAA ATC ATA AAG ATA TTG G</td>
<td>Fojer et al., 1994</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>HCO2198</td>
<td>TTA ACT TCA GGG TGA CCA AAA AAT CA</td>
<td></td>
</tr>
<tr>
<td>COI</td>
<td>Forward</td>
<td>SF1F</td>
<td>GAT CTG GTC TTT TAG GAA CAG GAT TCA</td>
<td>Newly designed using Primer3 (Untergasser et al., 2012), specific to <em>Chrysomallon squamiferum</em> n. sp.</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>SF1R</td>
<td>TGT GAG ATA CCA TTC CAA ATC ACG CAG</td>
<td></td>
</tr>
<tr>
<td>16S</td>
<td>Forward</td>
<td>16Sa</td>
<td>CGC CTG TTT ATC ATA AAC AT</td>
<td>Palumbi et al., 1991</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>16Sb</td>
<td>CCG GTC TGA ACT CAG ATC TCG</td>
<td></td>
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<tr>
<td>18S</td>
<td>Part 1</td>
<td>18Sa2.0</td>
<td>ATG GTT GCA AAG CTG AAA C</td>
<td>Whiting et al., 1997</td>
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<tr>
<td>18S</td>
<td>Part 2</td>
<td>18S1F</td>
<td>TAC ATG TTG GAT CTT GCC AGT AG</td>
<td>Ginibet et al., 1996</td>
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<tr>
<td>18S</td>
<td>Part 3</td>
<td>18S3F</td>
<td>GTT CGA TTC CGG AGA AGG A</td>
<td>Whiting et al., 1997</td>
</tr>
<tr>
<td>28S</td>
<td>Forward</td>
<td>28SSFF</td>
<td>AGT AAC GGC GAG TGA AGC GGG</td>
<td>Newly designed using Primer3 (Untergasser et al., 2012), specific to <em>Chrysomallon squamiferum</em> n. sp.</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>28SSFR</td>
<td>CGG TTT CAC GTC CTC TTG AAC TCT CTC</td>
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The holotype is deposited in the Natural History Museum, London (NHMUK) and paratypes are deposited in global museums with significant malacological collections. Institutional abbreviations are as follows:

- AMS: Australian Museum, Sydney, Australia
- ANSP: Academy of Natural Sciences of Drexel University, Philadelphia, USA
- CAMZM: University Museum of Zoology, Cambridge, UK
- FMNH: Field Museum of Natural History, Chicago, USA
- MNHN: Museum national d’Histoire naturelle, Paris, France
- MZUSP: Museum of Zoology, University of São Paulo
- NHMUK: Natural History Museum, London, UK
- NHMW: Museum of Natural History, Vienna, Austria
- NMNH: National Museum of Ireland – Natural History, Dublin, Ireland
- NMSA: KwaZulu-Natal Museum, Pietermaritzburg, South Africa
- NMW: National Museum of Wales, Cardiff, UK
- OUMNH.ZC: Zoological Collection, Oxford University Museum of Natural History, Oxford, UK
- RBINS: Royal Belgian Institute of Natural Sciences, Brussels, Belgium
- ROMIZ: Invertebrate Zoology Section, Department of Natural History, Royal Ontario Museum, Toronto, Canada
- SIO-BIC: Scripps Institution of Oceanography Benthic Invertebrate Collection, San Diego, California, USA
- SMNH: Swedish Museum of Natural History, Stockholm, Sweden
- UCMP: University of California Museum of Paleontology, Berkeley, California, USA
- UMIT: The University Museum, The University of Tokyo, Tokyo, Japan
- USNM: National Museum of Natural History, Smithsonian Institution, Washington DC, USA
- ZISP: Zoological Institute of Russian Academy of Sciences, St. Petersburg, Russia
- ZMB: Museum für Naturkunde, Berlin, Germany
- ZRC: Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore, Singapore
- ZSM: Zoologische Staatssammlung München, Munich, Germany

### Systematic Descriptions

#### Clade NEOMPHALINA McLean, 1990

**Superfamily NEOMPHALOIDEA McLean, 1981**

**Family PELTOSPIRIDAE McLean, 1989**

**Chrysomallon n. gen.**

*Chrysomallon*—Wareén et al., MS, GenBank AY163398 (*nomen nudum*).

*Type species*: *Chrysomallon squamiferum* *n. sp.*

*Etymology*: *Chrysomallon* (Greek), golden haired. The name is given in reference to the metal coating often found on the gastropod’s shell and sclerites, which includes pyrite, commonly known as fool’s gold. Gender neuter.

*ZooBank registration*: urn:lsid:zoobank.org:act:68B63A40-6404-4926-B460-2FC66A03E624

*Diagnosis*: Very large for Peltospiridae, up to 45.5 mm in maximum shell dimension. Coiled shell with three whorls; spire compressed; aperture elliptic. Periostracum thick. Foot large; epipodial tentacles covered by hundreds of hard dermal sclerites. Anterior pedal gland lacking. Oesophageal gland hypertrophied. Rest of digestive system reduced, with short intestine forming a simple loop. Cephalic tentacles thick at their bases.

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**Table 3. List of clade, family, species and genes used in the five-genes phylogenetic reconstruction with GenBank accession numbers.**

<table>
<thead>
<tr>
<th>Clade</th>
<th>Family</th>
<th>Taxa</th>
<th>COI</th>
<th>H3</th>
<th>16S</th>
<th>18S</th>
<th>28S</th>
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<tr>
<td>Patellogastropoda</td>
<td>Lottidae</td>
<td><em>Lottia gigantea</em> Gray in Sowerby I, 1834</td>
<td>FJ977750</td>
<td>FJ977725</td>
<td>FJ977696</td>
<td>FJ977632</td>
<td>AB282783</td>
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<td></td>
<td>Neolepetopsisae</td>
<td><em>Paralepetopsis</em> sp. SWA-2009 (from Aktipis &amp; Giribet, 2012)</td>
<td>FJ977725</td>
<td>FJ977728</td>
<td>FJ977699</td>
<td>FJ977635</td>
<td>FJ977665</td>
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<tr>
<td>Vetigastropoda</td>
<td>Fissurellidae</td>
<td><em>Fissurella barbadensis</em> (Gmelin, 1791)</td>
<td>HM771628</td>
<td>HM771595</td>
<td>HM771551</td>
<td>HM771467</td>
<td>HM771511</td>
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<td></td>
<td>Lepetodrilidae</td>
<td><em>Lepetodrilus elevatus</em> McLean, 1988</td>
<td>U56846</td>
<td>AY923959</td>
<td>EF549688</td>
<td>AY145381</td>
<td>AY145413</td>
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<td></td>
<td>Trochidae</td>
<td><em>Gibbula cineraria</em> (Linnaeus, 1758)</td>
<td>AM049339</td>
<td>FJ977737</td>
<td>AY163410</td>
<td>AY340430</td>
<td>FJ977676</td>
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<td>Cocculiniformia</td>
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<td><em>Cocculina messingii</em> McLean &amp; Harasewych, 1995</td>
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<td>AY923945</td>
<td>AY377624</td>
<td>AF120508</td>
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<td>Neomphalina</td>
<td>Melanodrymiidae</td>
<td><em>Melanodrymia aurantica</em> Hickman, 1984</td>
<td>U568736</td>
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<td>GQ160740</td>
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<td>GQ160805</td>
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<td>Neomphalidae</td>
<td><em>Cytheria naticoides</em> Warén &amp; Bouchet, 1989</td>
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<td>DQ093472</td>
<td>DQ093430</td>
<td>FJ977685</td>
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<td>Peltospiridae</td>
<td><em>Chrysomallon squamiferum</em> <em>n. sp.</em></td>
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<td>KP989447</td>
<td>KP989444</td>
<td>KP989445</td>
<td>KP989446</td>
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base, elongate, tapering towards tip. Ctenidium bicipitate, very large. No sexual dimorphism. Radula rhizodiglossate; formula $\sim 50 + 4 + 1 + 4 + \sim 50$; central tooth solid, strong with smooth cusps; lateral teeth strong with finely serrated cusps; marginal teeth long, slender, truncated, comb-like at distal end.

Remarks: As already discussed by Warén et al. (2003), within Neomphalina the available morphological information places Chrysomallon in the Peltospiridae. Morphologically, the primary distinguishing characteristic between the three neomphaline families is the nature of the copulatory appendages (Heß et al., 2008). Known species of Neomphalidae all have separate sexes and exhibit sexual dimorphism in which the left tentacle in males is modified and serves as a penis (Fretter, Graham & McLean, 1981; Beck, 1992; Heß et al., 2008). Members of Melanodrymidiae also have specialized copulatory organs in males of gonochoristic species (both tentacles in Melanodrymidiae males, Hazprunar, 1989; a large and ciliated swelling between cecal tentacles in Leptogryposis males, Heß et al., 2008) and in simultaneous hermaphrodites (additional copulatory appendages innervated by left cerebral ganglion, Heß et al., 2008). In Peltospiridae this is not the case and species do not have distinct copulatory organs or modifications of the cecal tentacles (Fretter, 1989; Israelsson, 1998). Chrysomallon likewise does not exhibit external sexual dimorphism and does not have copulatory appendages, which supports its placement in Peltospiridae. Chrysomallon also has a truncated comb-like ending to the marginal teeth, which is present in only the Peltospiridae and Melanodrymidiae. A series of bulges along ventral margin of the gill leaflets is another key characteristic separating Peltospiridae from the other groups and is present in Chrysomallon, as reported by Warén et al. (2003: fig. S2D).

Chrysomallon can easily be distinguished from all other described gastropod genera by having hundreds of dermal sclerites covering the foot, which is a unique characteristic (Warén et al., 2003). For Neomphalina, epipodial structure is an important anatomy of many peltospirid genera has been investigated, including Chrysomallon can easily be distinguished from all other described gastropod genera by having hundreds of dermal sclerites covering the foot, which is a unique characteristic (Warén et al., 2003). For Neomphalina, epipodial structure is an important characteristic (Ware´ n, 1989) and has a much more depressed spire, larger aperture (Mollusca NHM 110415); 2 species (MNHNIM 15:11-1-2); 2 species (FMNH 344546); 5 species (MNHN IM-2000-30072); 2 species (MZUSB 120572); 2 species (Mollusca NHM 110415); 2 species (MNHNIM 15:1:1-2); 2 species (NMNZ M.317895); 2 species (NSMA 19792/T4023); 2 species (NMNW Z.2015.005.00001-00002); 2 species (RBINS IG.32969/MT.3219); 2 species (ROMIZ M11286); 2 species (SIO-BIC M13766); 2 species (UMUT RMS/14); 13 species (UCMP 259000/1P9159); 2 species (USNM 127640); 2 species (ZISP 62033); 2 species (ZM Moll. 193973); 2 species (ZRC MOL.5793); 2 species (ZSM Mol. 20150039). Paratypes in 10% buffered formalin: Longgi vent field, Southwest Indian Ridge, 37°47.035’S, 49°38.960’E (’Tiamat Chimney’), 2783 m deep, RRS James Cook expedition JC67, ROV Kiel 6000 Dive 140, 27.11.2011, leg. J. T. Copley, 5 species (NHMUK 2015.0044); 20 species (UCMP 259001/1P9160); 1 spec., serially sectioned at 1.5 µm (ZSM Mol. 20151000).

Other material examined: c. 100 specimens, same collection data as holotype.

Eymology: squamiferum (Latin) meaning scale-bearing, referring to the numerous hard sclerites covering the foot. Used as an adjective.

Chrysomallon squamiferum n. sp.

Chrysomallon squamiferum—Warén et al., MS, GenBank AY163398 (nomen nudum).


The scaly snail—Goffredi et al., 2004: 3082.

The “scaly-foot” gastropod—Suzuki et al., 2006: 39.

Chrysomallon squamiferum—Yao et al., 2010: 987 (nomen nudum); missselling, A. Warén, personal communication).

Scaly-footed gastropods—Tao et al., 2012: 49.


Scaly-foot gastropods—Beedessse et al., 2013: 1.

Scaly-foot gastropod—Watanabe & Beedessse, 2015: 207.

Chrysomallon squamiferum—Nakagawa et al., 2014: 40 (nomen nudum); missselling, A. Warén, personal communication).

The ‘scaly-foot gastropod’—Chen et al., 2013: 950.

Type material: Holotype (shell diameter 32.03 mm, 90% ethanol). Longgi vent field, Southwest Indian Ridge, 37°47.035’S, 49°38.960’E (’Tiamat Chimney’), 2783 m deep, RRS James Cook expedition JC67, ROV Kiel 6000 Dive 140, 27.11.2011, leg. J. T. Copley (NHMUK 2015.0041). Paratypes in 99% ethanol (collection data as holotype): 1 dissected spec., shell diameter 36.03 mm (NHMUK 2015.0042); growth series of 5 specs (NHMUK 2015.0043); growth series of 5 specs (OUMNH.ZC 2013.02.001); growth series of 5 specs (SMNH Type Colln 8449); 2 specs (AMS C.483502); 2 specs (ANSP A23851); 2 specs (CMAMZ 2015.1.1-2); 2 specs (FMNH 344546); 5 species (MNHN IM-2000-30072); 2 species (MZUSB 120572); 2 species (Mollusca NHM 110415); 2 species (MNHNIM 2015.1.1-2); 2 species (NMNZ M.317895); 2 species (NSMA 19792/T4023); 2 species (NMNW Z.2015.005.00001-00002); 2 species (RBINS IG.32969/MT.3219); 2 species (ROMIZ M11286); 2 species (SIO-BIC M13766); 2 species (UMUT RMS/14); 13 species (UCMP 259000/1P9159); 2 species (USNM 127640); 2 species (ZISP 62033); 2 species (ZM Moll. 193973); 2 species (ZRC MOL.5793); 2 species (ZSM Mol. 20150039). Paratypes in 10% buffered formalin: Longgi vent field, Southwest Indian Ridge, 37°47.035’S, 49°38.960’E (’Tiamat Chimney’), 2783 m deep, RRS James Cook expedition JC67, ROV Kiel 6000 Dive 140,

Description: Shell (Fig. 2C, D). Three whorls, globose with depressed spire, tightly coiled. Milky white, thin. Aperture ellipctic, very large. Shape between neritiform and limpet-form. Surface sculptured with subtle, closely-spaced ribs. Fine growth lines present. Periostracum thick, brown, envelopes shell edge. Exterior often coated by a black layer of iron sulphide. Columellar region covered by thin callus. Average shell diameter 32 mm for adults (100 specimens), maximum shell diameter 45.5 mm. Protoconch entirely into shell, red when alive. Sole of foot surrounded by eyes or pigment. Snout tapered, thick. Foot large, unable to contract entirely into shell, red when alive. Sole of foot surrounded by pedal flange. Epipodium without epipodial tentacles; covered by hundreds of hard sclerites. As discussed by Warén et al. (2003), shell muscle large, horeshoe shaped, with two anterior parts on each side connected by a narrow connective posteriorly.

Operculum. Present in metapodium buried under layers of sclerites (Chen et al., 2013: fig. 2A). Multispiral, concentric in juveniles. Shifts to curved, bent shape in adult (Chen et al., 2015: fig. 2C).
Figure 2. *Chrysomallon squamiferum* n. sp. A, B. Live specimen from Longqi vent field, SWIR. C, D. Paratype (NHMUK 2015-0042). E, F. Holotype (NHMUK 2015-0041). G. Close-up of sclerites. H. In situ photograph, Longqi vent field, SWIR. Scale bars: A, B, G = 1 cm; C–F = 5 mm; H = 5 cm. Photograph credits: A, B, David Shale; C–F, Pete Bucktrout (British Antarctic Survey).
Sclerites (Figs 2E–G, 3). Curved, elongate, proteinaceous, not calcified. Approximately 1 x 5 mm in size in adults. White to metallic black depending on extent of iron sulphide coating; newly grown sclerites milky white. Ferrimagnetic when coated with iron sulphide layer.

Internal anatomy (Fig. 4). As discussed by Warén et al. (2003). Ctenidium bipectinate, very large. Ventral margin of gill leaflet carries series of bulges (Warén et al., 2003: fig. S2D). Vascular system hypertrophied. Heart monotoocardian with discrete auricle and ventricle. Oesophageal gland hypertrophied. Rest of digestive system relatively small, in particular intestine, midgut and stomach. Rectum exits to mantle cavity on right. Gonads displaced anteriorly to headfoot region, occupying ventral face of visceral mass. ‘Spermatophore packaging organ’ present; spermatophores known and described by Warén et al. (2003: fig. S2M). Genital slit simple, opening to mantle cavity on right ventral of rectum. No sexual dimorphism observed. Digestive gland occupies dorsal face of visceral mass into apex. Interconnected pedal nerves large, conspicuous under oesophageal gland. Anterior pedal gland absent.

Radula (Fig. 5). Rhipidoglossate. Ribbon approximately 0.5 mm wide and 4 mm long in adults. No difference between anterior and posterior rows in radulae investigated, showing no signs of wear. General appearance of radula typical of family; formula ~50 + 4 + 1 + 4 + ~50. Cusp of central tooth blunt-ended, not crenulated. Laterals solid with reinforced bases, cusps blunt ended, inner and outer side very finely crenulated. Marginal teeth elongate with a truncated distal end dividing into about 20 slender denticles.

**Distribution:** Known from the following three hydrothermal vent fields in the Indian Ocean, found on active black smokers as well as on diffuse flow sites. Longqi vent field (37°47.027’S 49°38.963’E), SWIR, around 2,780 m depth; Kairei vent field (25°19.239’S, 70°02.429’E), Central Indian Ridge, depth 2,415 to 2,460 m (Van Dover et al., 2001); and Solitaire vent field (19°33.413’S, 65°50.888’E), Central Indian Ridge, depth 2,606 m (Nakamura et al., 2012).

**Remarks:** The shell parameters and proportions are summarized in Table 4. Figure 6 shows a scatterplot of shell diameter against shell height across life stages (shell diameter range 9.80–40.02 mm); the regression shows a significantly linear relationship ($R^2 = 0.9869, P < 0.001$). The spire apices investigated were extremely brittle and severely corroded from the outside, so that no useful information was available on the protoconch. The above description is based on specimens from the type locality: Longqi vent field, SWIR. This locality was chosen as type locality because of the large number of specimens available. Warén et al. (2003) published many relevant observations made from specimens collected from the Kairei vent field, CIR, which clearly belong to the same species, based on genetic support (see below) and similar morphology. From published figures, specimens from Kairei and Solitaire vent fields in CIR appear to have broader sclerites than those from Longqi (cf. Fig. 2E; Warén et al., 2003: fig. S2E; Nakamura et al., 2012: fig. 5), but all are otherwise indistinguishable. The specimens from the Solitaire vent field are not covered in iron sulphide and thus have white sclerites and a light brown shell resulting from the exposed periostracum (Nakamura et al., 2012: fig. 5B). Examined specimens from SWIR reached a maximum size of 40 mm in shell diameter, whereas from Kairei, CIR, they are known to reach a maximum of 45.5 mm (A. Warén, personal communication). The operculum is known from all populations and is similar in each (Chen et al., 2015).

**Genetic analyses**

The COI consensus tree constructed with five randomly selected haplotypes from each of the three populations of Chrysomallon...
Figure 4. Internal anatomy of *Chrysomallon squamiferum* n. sp. drawn with shell, mantle and part of ctenidium removed. Oesophageal gland and gonad are partly dissected to show structures underneath or within. Abbreviations: Au, auricle; Ct, ctenidium; Dg, digestive gland; Go, gonad; Gs, genital slit; In, intestine; Mo, mouth; Ne, nephridium; Oe, oesophagus; Og, oesophageal gland; Re, rectum; Sc, sclerite; Sp, ‘sperm packaging organ’; Ve, ventricle. Scale bar = 1 cm.
Chrysomallon squamiferum is shown in Figure 7. Chrysomallon squamiferum is a discrete lineage within Peltospiridae and the three populations are closely similar. The average pairwise distance of Longqi specimens from Kairei (average of five specimens 0.74%, range 0.35–0.98%) and Solitaire specimens (average of five specimens 0.86%, range 0.39–1.38%) was 0.80%, while that between Kairei and Solitaire specimens was 0.88% (average of five specimens, range 0.19–1.58%). These differences are relatively small and are within the intraspecific range (interspecific differences for COI in marine gastropods commonly exceed 4%; e.g. Meyer & Paulay, 2005) and are consistent with the interpretation that the three populations belong to a single species. It has been previously indicated that the COI gene does not provide enough resolution to clarify the internal relationships of the Neomphalina (Aktipis & Giribet, 2012). The purpose of this analysis was to demonstrate the differentiation among the populations of C. squamiferum, so only peltospirid sequences were included.

A maximum-likelihood distance matrix constructed from COI sequences of six Peltospiridae genera including Chrysomallon is shown in Table 5. All species used are the type species of their respective genera, except Nodopelta for which COI sequences of the

Table 4. Shell parameters of Chrysomallon squamiferum n. sp. Range and proportion to shell diameter calculated from 50 specimens across a size range.

<table>
<thead>
<tr>
<th>Shell parameters (mm)</th>
<th>Aperture parameters (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diameter</strong></td>
<td><strong>Height</strong></td>
</tr>
<tr>
<td>Holotype (NHMUK 2015-0041)</td>
<td>32.03</td>
</tr>
<tr>
<td>Paratype (NHMUK 2015-0042)</td>
<td>36.03</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td><strong>9.80–40.02</strong></td>
</tr>
<tr>
<td><strong>Proportion to shell diameter</strong></td>
<td>–</td>
</tr>
<tr>
<td><strong>SD of proportion</strong></td>
<td>–</td>
</tr>
</tbody>
</table>
type species *N. heminoda* McLean, 1989 were not available and therefore *N. subnoda* McLean, 1989 was used instead. The pairwise COI divergence between representatives of the five described peltospirid genera averages 20.81% (range 13.65–24.68%), while their divergence from *Chrysomallon* (Longqi vent field) averages 25.34% (range 24.20–26.41%). This is consistent with the generic rank of *Chrysomallon*.

The consensus tree from the five-gene Bayesian phylogenetic analyses is shown in Figure 8. *Chrysomallon squamiferum* fell within a marginally supported Peltospiridae clade (posterior probability, PP = 80%) which was sister to *Cyathermia naticoides* (Neomphalidae). The clade Neomphalina was recovered with full support (PP = 100%). These results do not contradict with the placement of *C. squamiferum* in Peltospiridae as indicated by morphological characteristics.

**DISCUSSION**

*The scientific name*

Since its discovery, the biology of *Chrysomallon squamiferum* n. sp. and its position as a representative endemic species of Indian Ocean hydrothermal vents has attracted interest from the scientific community as well as the public. Numerous papers have been published on various aspects of the biology and ecology of this species (e.g. sclerites and anatomy, Waren et al., 2003; endosymbionts, Goffredi et al., 2004; sclerite biomineralization, Suzuki et al., 2006; shell biomineralization, Yao et al., 2010). However, in the absence of a formal name, each publication has used a slightly different version of ‘scaly-foot gastropod’ or the
Table 5. Maximum-likelihood distance matrix of seven Peltospiridae genera constructed from 457 bp fragment of COI gene.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chrysomallon squamiferum n. sp. (Longqi, SWIR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Chrysomallon squamiferum n. sp. (Kairei, CIR)</td>
<td>0.66%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Chrysomallon squamiferum n. sp. (Solitaire, CIR)</td>
<td>0.66%</td>
<td>0.88%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Depressigyna globulus</td>
<td>26.41%</td>
<td>26.15%</td>
<td>26.41%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Nodopeita subnoda</td>
<td>24.46%</td>
<td>23.83%</td>
<td>24.16%</td>
<td>17.73%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Pachydermia laevis</td>
<td>25.48%</td>
<td>25.74%</td>
<td>25.49%</td>
<td>24.05%</td>
<td>21.29%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Peltospira operculata</td>
<td>24.20%</td>
<td>24.24%</td>
<td>24.49%</td>
<td>23.31%</td>
<td>17.10%</td>
<td>24.08%</td>
</tr>
<tr>
<td>8</td>
<td>Rhynchopelta concentrica</td>
<td>26.14%</td>
<td>26.10%</td>
<td>26.14%</td>
<td>22.94%</td>
<td>13.65%</td>
<td>24.68%</td>
</tr>
</tbody>
</table>

Analyses were conducted using the Maximum Composite Likelihood model (Tamura, Nei & Kumar, 2004). Chrysomallon squamiferum n. sp. sequences represent one single specimen.

Figure 8. Consensus tree reconstructed using Bayesian inference from a five-gene (H3, COI, 16S, 18S, 28S) 2753-bp dataset including all available members of Neomphalina. Node values are Bayesian posterior probability.

misspelt manuscript name and nomen nudum ‘Crysomallon squamiferum’ (see synonymy above) to address the species. The name formally assigned here is the same as the original manuscript name by Ware´n et al. (2003, used in original GenBank registration AY163398). This name is here validated according to the requirements of the International Code of Zoological Nomenclature (ICZN, 1999) and should obviate further confusion with regard to this taxon.

Distribution across two mid-ocean ridges

Both morphological and genetic results are consistent with the conclusion that the population newly discovered in Longqi vent field, SWIR represents the same species as the previously known populations in the Kairei and Solitaire fields, CIR. This extends the distribution of C. squamiferum across the 2,500 km distance between Longqi and Solitaire. The Longqi population has black sclerites and shell like the Kairei population, although the sclerite is more elongate, while the Solitaire population has white sclerites and brown shell because of a lack of iron sulphide (Nakamura et al., 2012). This is likely to result from the lack of iron in the vent fluid of Solitaire (Nakamura et al., 2012). Similarly, a number of neomphalines (e.g. Linapex, Pachydermia, Melanodrymia) have been reported to have rusty deposits (likely deposited by bacteria) or to lack them, depending on the environment (Ware´n & Bouchet, 2001).

Although the SWIR and CIR populations are genetically similar, the question as to whether connectivity is maintained over the intervening distance needs further investigation. Two studies (Nakamura et al., 2012; Beedessee et al., 2013) have shown that connectivity is high between two populations of scaly-foot gastropods on the CIR at the Kairei and Solitaire vent fields,
approximately 700 km apart. Although 2,500 km is not a particularly long distance for vent species to maintain connectivity (e.g., vent shrimps *Rhinocaris exoculata* with planktotrophic larvae have been shown to maintain high connectivity over 7,000 km of Mid-Atlantic Ridge; Teixeira, Serr O & Arnaud-Haond, 2006) the dispersal ability of *C. squamiferum* may be low as it most likely has lecithotrophic development (presumably with a planktonic dispersal stage like all other known peltospirids; Warén, Bouchet & Cisler, 2006) and negatively buoyant eggs (Beedessee et al., 2013). Across the East Pacific Rise the vent limpet *Lepetodrilus elevatus*, also with lecithotrophic development, was shown to exhibit significant genetic differentiation (Poulyez et al., 2009). The new population at Longqi provides an opportunity for a future study of connectivity between hydrothermal vents across different mid-ocean ridges, for which there is currently no knowledge within the Indian Ocean. Interestingly a number of active vents have been inferred between Longqi and Kairei (e.g. SWIR 63.9°E, Tao et al., 2009; SWIR 58.9°E, German et al., 1998) and it is likely that these and this further unexplored vent fields could act as stepping stones between Longqi and CIR vents to maintain connectivity.

**Acknowledgements**

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**References**


