A new genus of large hydrothermal vent-endemic gastropod (Neomphalina: Peltospiridae)

Short running title

New genus of vent-endemic neomphalin gastropod

Abstract

Recently discovered hydrothermal vent fields on the East Scotia Ridge (ESR, 56-60°S 30°W), Southern Ocean and the South West Indian Ridge (SWIR, 37°S 49°E), Indian Ocean, host two closely related new species of peltospirid gastropods. Morphological and molecular (mitochondrial cytochrome c oxidase subunit I, COI) characterisation justify the erection of *Gigantopelta* gen. nov. within the Peltospiroidae with two new species *Gigantopelta chessoia* sp. nov. from ESR, and *Gigantopelta aegis* sp. nov. from SWIR. They attain an extremely large size for the clade Neomphalina, reaching 45.7mm in shell diameter. The esophageal gland of both species markedly enlarged. *G. aegis* has a thick sulphide coating on both the shell and the operculum of unknown function. The analysis of a 579bp fragment of the COI gene resulted in 19-28% pairwise distance between *Gigantopelta* and six other genera in Peltospiridae, while the range among those six genera was 12-28%. The COI divergence between the two newly described species of *Gigantopelta* was 4.43%. Population genetics analyses using COI (370bp) of 30 individuals of each species confirms their genetic isolation and indicate recent rapid demographic expansion in both species.

25

26 **Additional Keywords**

27 *Gigantopelta*, East Scotia Ridge, Indian Ocean, Southern Ocean, population genetics

Introduction

Gastropods are an important component of the fauna of hydrothermal vents in terms of abundance and biomass. In some cases, they are amongst the dominant megafaunal groups that characterise vent biogeographic provinces e.g., *Alviniconcha hessleri* Okutani & Ohta, 1988 and *Ifremeria nautilei* Bouchet & Warén, 1991 which dominate the west Pacific vents in the Manus, Fiji and Lau Basins. More than 218 gastropod species have been described from chemosynthetic ecosystems, of which more than 138 are believed to be endemic to these ecosystems (Sasaki *et al.*, 2010).

In 2010, the British expedition JC42 on board RRS *James Cook* sampled the hydrothermal vents at East Scotia Ridge (ESR) for the first time, discovering a hitherto unknown species of gastropod (Rogers *et al.,* 2012). This large gastropod was one of the dominant megafaunal taxa along with an undescribed species of yeti crab of the genus *Kiwa*, and the recently described eolepadid stalked barnacle *Vulcanolepis scotiaensis* Buckeridge, Linse & Jackson, 2013. Marsh *et al.,* (2012: Fig. 2A) reports zonation patterns in hydrothermal vents of the E9 segment of ESR, where different animals dominate different zones according to distance from vent fluid exit. The area closest to fluid exit is dominated by three size classes of *Kiwa*, followed by multilayer assemblages of the large gastropod, then *Vulcanolepis scotiaensis*, and finally actinostolid anemones before the vent periphery zone. The gastropod species was identified to be a member of the superfamily Neomphaloidea (as Peltospirioidea) in the clade Neomphalina (Rogers *et al.,* 2012).

Figure 1

In 2011, another British expedition, RRS *James Cook* JC67, surveyed the first-known vent field on the Southwest Indian Ridge (SWIR), the Longqi (previously also known as 'Dragon'; Roterman *et al*., 2013) vent field (Tao *et al*., 2014). This expedition yielded another peltospirid gastropod, morphologically closely resembling the species discovered in ESR. This latter species was one of the dominant taxa, forming dense aggregations mostly in areas of diffuse flow of vent fluids (Fig. 2B).

Figure 2

Neomphalina (Warén & Bouchet, 1993) is a clade of gastropods entirely endemic to chemosynthetic environments (Sasaki *et al.,* 2010). The monophyly of this clade has been well supported by molecular studies (McArthur & Koop 1999; Warén *et al.,* 2003; Aktipis *et al.,* 2008; Aktipis & Giribet, 2010; 2012) but the morphology is very diverse between members so that morphological characterisation is difficult (Sasaki *et al.,* 2010). The Neomphalina comprise the superfamily Neomphaloidea which contains the families Melanodrymiidae, Neomphalidae and Peltospiridae. The internal relationships between these three families are unresolved even with molecular methods, as some studies support monophyly of the families (e.g., Heβ *et al.,* 2008) while others do not (e.g., Aktipis & Giribet, 2012). The position of this clade in the broader scheme of gastropod systematics is still very much in debate, partly because of this morphological variability (Sasaki *et al.,* 2010). Most recent molecular phylogenies place Neomphalina basal to Vetigastropoda, with Cocculinoidea as sister clade (Aktipis & Giribet, 2012).

The aim of the present study is to describe the morphology and genetic characterisation of the two species and to assess their status within the clade Neomphalina. As the two species are very closely related, population genetic methods are used to provide insights

into their diversification.

Materials & Methods

East Scotia Ridge

Following the initial discovery of hydrothermal vent sites on E2 (56°05.31'S 30°19.10'W) and E9 (60°03.00'S 29°58.60'W) segments of the ESR in 2009 on RRS *James Clark Ross* expedition JR224, vent fauna from these sites were collected during RRS *James Cook* expedition JC42 in the austral summer of 2011 using the remotely operated vehicle (ROV) *Isis* (Rogers *et al.,* 2012). Specimens of a large brown peltospiroid were collected using the suction sampler or scoop by the ROV *Isis* and either fixed in 96% pre-cooled ethanol or 4% buffered formaldehyde or frozen at -80^{o}C upon recovery. They were stored cooled or frozen until dissection or DNA extraction.

South West Indian Ridge

The Longqi vent field (37°47.03'S 49°38.96'E; Tao *et al*., 2014) was confirmed by the Chinese RV *Da Yang Yi Hao* expedition DY115-19 in 2007 (Tao *et al.,* 2012) and is the first visually-confirmed hydrothermal vent field on the Southwest Indian Ridge. This site was first sampled during the RRS *James Cook* expedition JC67 in 2011, and has previously been referred to as the Dragon vent field (Roterman *et al*., 2013). Specimens of another large peltospirid were collected using the suction sampler of ROV *Kiel 6000*

and fixed in 10% buffered formalin for morphological examination and in 96% ethanol for genetic studies.

Morphology

External morphological investigation and dissection were carried out with a Leica 10x magnification dissection microscope. The radulae were dissected from specimens preserved in 100% ethanol or frozen and prepared for Scanning Electron Microscopy (SEM) using the following protocol. Tissues around the radula were dissolved with 10% KOH solution overnight. In large specimens, the area around the protoconch was dissected out to fit on SEM stubs, in small specimens, the entire shell was used. To clean before drying, samples underwent a hydration series in 75% - 60% - 40% - 20% - 0% ethanol solution, each step lasting 15 minutes and ending in a rinse in distilled water. Sonication in distilled water was carried out with a single drop of TWEEN 80 for 10 seconds followed by rinsing in distilled water for 15 minutes. The samples then underwent dehydration series in 0% - 20% - 40% - 60% - 75% ethanol solution, each step lasting 15 minutes. At the end of washing samples were rinsed in 100% ethanol for 15 minutes and then stored in fresh 100% ethanol. Washed specimens were dried completely using hexamethyldisilazane for 1-5 minutes and then air-dried overnight. After mounting on SEM stubs with carbon disks samples were coated with gold using a Quorum Technologies E5000 sputter coater. SEM imaging was undertaken using a Jeol JSM-5510 SEM (Department of Plant Sciences, University of Oxford). Specimens for protoconch investigation were dried and mounted in the same manner.

Soft parts were drawn using pencil with the aid of a Zeiss Stemi SV6 microscope mounted with a Zeiss camera lucida drawing tube, and then traced with a black pen. The image was digitised by a HP Photosmart 2575 scanner at resolution of 600dpi and post-processed using Adobe Photoshop CS6.

Shell morphometric measurements were carried out using digital vernier callipers.

Genetics

For all genetic analyses, individuals collected from Segment E2, ESR and Longqi vent field, SWIR were used. Partial sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene, 579-bp in length, were used to check the sequence identity of the discovered peltospiroid species against other known species of Neomphalina. *Cocculina messingi* (Cocculinoidea) was used as an outgroup.

Genomic DNA was extracted from foot tissue using QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's instructions (Crawley, West Sussex, United Kingdom), and extractions were stored in -20 ^{o}C freezers. Quality of the DNA was assessed using a Nanodrop 2000 spectrophotometer.

The COI region of the ESR peltospiroids was amplified with the primer pair LCO1490 and HCO2198 (Folmer *et al.,* 1994). Amplification of COI from the SWIR peltospirid required the design of the following primer pair from Peltospiridae COI sequences on

GenBank using Primer3 (Rozen & Skaletsky, 2000) and resulted in a high success rate. These new primers are designated as:

SB1F (5'- AGCCGTGTTGAAATTACGGTCAGT -3')

And

SB1R (5'- GTCTGCTTTACTGGGGACAGG -3').

This set of primers amplified an approximately 480bp fragment of COI.

The polymerase chain reaction was carried out in 12µl reaction volumes, including 2µl DNA template (100-200 ng/µl), 8µl QIAGEN Master Mix, 0.4µl double-distilled water, 1.6µl primer mix containing 0.8µl each of forward and reverse primers at concentrations of 4pmol/µl. Thermocycling was performed using a Bio-Rad C1000 Thermal Cycler, with the following protocol: initial denaturation at 95 ^{o}C for 15 minutes followed by 40 cycles of [denaturation at 94^{o}C for 45 seconds, annealing at 45^{o}C for 60 seconds, extension at 72^{o}C for 60 seconds], ending with final extension at 72^{o}C for 5 minutes. Amplification of the desired region was confirmed with 1% agarose gel electrophoresis with ethidium bromide. Successful PCR products were purified using either QIAGEN QIAquick PCR purification kit or Diffinity RapidTip, both using standard protocols.

Cycle sequencing reactions were carried out in 10µl volumes, containing 0.5µl BigDye Terminator v3.1 (Applied Biosystems), 2.5µl 5x buffer, 2.5µl PCR product, 2.5µl primer (0.8pmol/µl), 2µl double-distilled water. The following protocol was used: initial denaturation at 96^{o}C for 1 minute followed by 25 cycles of [denaturation at 96^{o}C for 10 seconds, annealing at 50^{o}C for 5 seconds, extension at 60^{o}C for 4 minutes], ending with final extension at 60^{o}C for 4 minutes. Sequenced products were precipitated using the

EDTA/ethanol method. Sequences were resolved from precipitated products using Applied Biosystems 3100 DNA sequencer (Sequencing Department, Department of Zoology, University of Oxford).

Alignment and editing of genetic sequences were carried out using the software Geneious 5.6 (Drummond *et al.,* 2011), and reads were manually quality-checked and corrected by eye. Only sequences with both good quality matching forward and reverse reads were used in downstream analyses. Pairwise distances of COI were calculated with software MEGA 5.05 (Tamura *et al.,* 2011). Prior to phylogenetic analyses, the most suitable evolutionary model was selected, using the Akaike Information Criterion in PartitionFinder v1.0.1 (Lanfear *et al.,* 2012). This selected the GTR + I + G model for all codon positions. Tree reconstruction was carried out with Bayesian inference using program MrBayes 3.2 (Ronquist *et al.,* 2012). The total aligned sequence length used in the analyses was 579bp. In the analysis, Metropolis-coupled Monte Carlo Markov Chains were run for five million generations. Topologies were sampled every 100 generations, and the first 25% were discarded as "burnin" to ensure chains had converged.

Population genetic inferences were made from the sequences of 30 specimens from each species using the software Arlequin v3.5.1.3 (Excoffier & Lischer, 2010). The same software was used for mismatch distribution analyses. The length of the COI sequences used in the population genetic analyses was 370bp as some specimens only had high-quality readings of this length. Haplotype diversity (h), nucleotide diversity (π) and pairwise F_{ST} were calculated, and the statistical significance of F_{ST} was calculated. Departures from equilibrium as expected for neutral markers were tested statistically

using Tajima's *D* test (Tajima, 1989) and Fu's F_S test (Fu, 1997) in the same program, using 10,000 permutations. Statistical parsimony networks were constructed using the software TCS v1.21 (Clement *et al.,* 2000) with the connection probability set to 95%.

New COI sequences generated from this study and used for population genetics analyses are deposited in GenBank under accession numbers XXYYYYYY-XXYYYYYY (*Gigantopelta chessoia* sp. nov.) and XXYYYYYY-XXYYYYYY (*Gigantopelta aegis* sp. nov.) (Table 1).

Table 1

Type specimens are deposited in the invertebrate collection at the Natural History Museum, London (NHMUK), the Zoological Collection of the Oxford University Museum of Natural History (OUMNH.ZC) and the Swedish Museum of Natural History (SMNH).

Results

Systematics

Clade NEOMPHALINA McLean, 1990

Superfamily NEOMPHALOIDEA McLean, 1981

Family PELTOSPIRIDAE McLean, 1989

***GIGANTOPELTA* gen. nov.**

Type species. *Gigantopelta chessoia* sp. nov., by original designation.

Etymology. Giganteus (Latin), gigantic; Pelta (Latin), shield. This refers to the extremely large adult shell size of the species in this genus for the family Peltospiridae. The genus name is feminine.

Diagnosis. Shell extremely large for family, reaching 45mm in adult shell length. Shell globose, rather loosely coiled with deep suture, 3-4 whorls. Spire depressed. Protoconch consisting of 0.5 whorls. Aperture very large, circular, expanding rapidly. Thick, dark olive periostracum enveloping edge of aperture. Shell milky white and thin, not nacreous. Columellar folds lacking. Concentric, multispiral operculum present. Foot large. Cephalic tentacles thick, broad, triangular, thinning towards tips. Eyes lacking. Snout tapering and thick. Esophageal gland hypertrophied. Single, bipectinate ctenidium. Sexes separate. Epipodial tentacles present surrounding operculum. Radula rhipidoglossate, formula ~ 50 + 4 + 1 + 4 + ~ 50. Central, lateral teeth strong, solid with smooth cusps. Marginal teeth long, slender, truncate, divided to about 20 toothlets to distal end.

Remarks. Adult *Gigantopelta* are easily distinguished from all other described peltospirids by their extremely large shell size. Furthermore, *Gigantopelta* can be distinguished from the limpet-like peltospirid genera *Ctenopelta* Warén & Bouchet, 1993*, Echinopelta* McLean, 1989*, Hirtopelta* McLean, 1989*, Nodopelta* McLean, 1989, and *Rhynchopelta* McLean, 1989 by having a coiled shell with 3-4 whorls. It can be distinguished from the three skeneiform genera, *Pachydermia* Warén & Bouchet, 1989, *Depressigyra* Warén & Bouchet, 1989 and *Lirapex* Warén & Bouchet, 1989, by its inflated form with a much more depressed spire and larger aperture. The shell surface is

nearly smooth, which differs from all peltospirid genera except *Depressigyra*. The shell roughly resembles that of *Peltospira*, but has a more tightly coiled initial whorl, and lacks lamellar sculpture. Analysis of the soft parts shows an enlarged esophageal gland, a feature previously only known from the yet undescribed 'scaly-foot gastropod' (Warén *et al.,* 2003), which is also the only other known peltospirid to attain a similar size. In the 'scaly-foot gastropod' the esophageal gland houses symbiotic bacteria, but it is unclear whether this is also the case for *Gigantopelta*. *Gigantopelta* can be distinguished from the 'scaly-foot gastropod' easily as it does not possess dermal sclerites, has a large operculum, and a shell that is less vertically compressed, with a more circular aperture. Shell of *Gigantopelta* may be coated in a layer of sulphide, which is frequent among vent gastropods including the neomphalins (Hickmann, 1984; Warén and Bouchet 2001). *Gigantopelta* is also comparable to the Oligocene fossil genus *Elmira* Cooke, 1919 from a seep deposit near Bejucal, Cuba; whose possible affinity to Neomphalina based on resemblance to the 'scaly-foot gastropod' has been remarked by Kiel & Peckmann (2007). Although the type species *Elmira cornuarietis* Cooke, 1919 is approximately the same size as *Gigantopelta* (> 40mm in shell length), it carries broad revolving grooves which *Gigantopelta* lack. The true taxonomic affinity of *Elmira* is still unclear.

***Gigantopelta chessoia* sp. nov.** (Figs. 2-7)

'Peltospiroidea n. sp.' – Rogers *et al.*, 2012: 7, Fig. 3D

'Undescribed species of peltospiroid gastropod' – Marsh *et al.*, 2012: 6, Fig. 5C, 5J.

Type material: Holotype. Shell diameter 36.30 mm, 99% ethanol, Fig. 3A-C. E2 segment, East Scotia Ridge, 56°05.31'S 30°19.10'W ('Cindy's Castle'), 2606 m deep, RRS *James Cook* expedition JC42, ROV *Isis* Dive 130, 20.01.2010, leg. A. D. Rogers (NHMUK 2015.XX). Paratypes. One dissected specimen, 99% ethanol (shell diameter 31.12mm, Fig. 4A-B; NHMUK 2015.XX); growth series of five specimens, 99% ethanol (NHMUK 2015.XX). The above two lots have same collection data as holotype. Growth series of five specimens, 99% ethanol (OUMNH.ZC.2013.02.002); growth series of give specimens, 99% ethanol (SMNH Type Collection 8450); five specimens, 10% buffered formaldehyde (NHM 2015.XX). Collection data for the latter three lots: E2 segment, East Scotia Ridge, 56°05.34'S 30°19.07'W ('Cindy's Castle'), depth 2644 m, RRS *James Cook* expedition JC42, ROV *Isis* Dive 134, 24.01.2010, leg. A. D. Rogers.

Materials Examined: Approximately 200 specimens collected on RRS *James Cook* expedition JC42 with ROV *Isis*, on dives 130, 134 and 141. Collection data for dive 130: same as holotype; dive 134: same as listed for paratype series; dive 141: E9 Segment, East Scotia Ridge, 60°02.81'S 29°58.71'W ('Marsh Tower'), depth 2394 m, RRS *James Cook* expedition JC42, ROV *Isis* Dive 141, 30.01.2010, leg. A. D. Rogers.

Etymology: The species is named after the ChEsSO Consortium, under which ESR hydrothermal vents and this species were discovered.

Figure 3

Figure 4

Description / Diagnosis:

Shell: Shell (Fig. 4A-B) globose, 3-4 whorls, coiled tightly with a deep suture. Spire

depressed. Aperture roughly circular, very large. Ratio of shell diameter to aperture length approximately 1:0.633 (average of 100 specimens). Shell trochiform to neritiform, holostomous. Protoconch (Fig. 5A) consists of 0.5 whorls, diameter about 210 μm. Irregular reticulate ornament present initially, becoming obsolete distally. Suture around protoconch very deep. Teleoconch smooth, no distinct sculpture. Subtle growth lines, irregular protuberances present. Growth lines stronger on the body whorl, especially near the aperture. Periostracum thick, dark olive, enveloping the aperture. Ostracum and hypostracum milky white. Thin, fragile without periostracum. Columellar folds lacking. Callus extends over just covering columellar. Area around callous concave. Maximum shell diameter 45.7mm.

Operculum: Operculum (Figs. 3C) with central nucleus, multispiral, thin, flaky on fringe. Operculum fringe often damaged. Juveniles operculum thin, semi-transparent, fringe not flaky (Fig. 5C).

Figure 5

Radula: Radula (Fig. 6A) rhipidoglossate. Ribbon approximately 0.5 mm wide and 4 mm long in adults. Formula ~ 50 + 4 + 1 + 4 + ~ 50. Central, lateral teeth cusp-like, pointed (Fig. 6C). Marginal teeth long, slender, bearing ~ 20 denticles at distal end (Fig. 6E). Central tooth triangular, very broad at base, tapering distally, smooth, no sculpture. Lateral teeth solid, bearing a clear protrusion at base.

Soft parts (Fig. 7A): Foot muscular, large. Fully retractable into shell, red when alive.

Figure 6

Small epipodial tentacles present, surrounding posterior 2/3 of operculum. Cephalic tentacles thick, triangular, broad at base and thinning towards tips. Eyes lacking. Snout

315 tapering, thick. Esophageal gland huge, approximately same size as aperture. Ctenidium
316 bipectinate. Sexes separate. Shell muscle large, horse-shoe shaped. Intestine forms a
317 simple loop.
318
319 *Distribution:* Only known from hydrothermal vents on segment E2 (56°05.2'S to
320 56°05.4S, 30°19.00'W to 30°19.35'W) and E9 (60°02.50'S to 60°03.00'S, 29°58.60'W to
321 29°59.00'W) of the East Scotia Ridge. This species forms dense aggregations rather close
322 to vent effluents.
323
324 *Remarks*: The dispersal mechanism is inferred to be non-planktotrophic from the
325 protoconch, presumably with a planktonic dispersal stage. Table 2 shows the shell
326 parameters of *G. chessoia*. The relationships between the six shell parameters measured
327 were investigated and they were all linear across all life stages. Fig. 8 shows a scatterplot
328 of shell diameter against shell height. See Rogers *et al.,* (2012) for details on location of
329 hydrothermal vent sites.
330
331 *Comparative remarks:* Similar to *Gigantopelta aegis* sp. nov. described below. *G.*
Table 2
332 *chessoia* can be distinguished as it has a taller spire, less extensive callus, and area around
333 callus being concave and not flattened as in *G. aegis*. Difference is seen in the structure of
334 the radula. The central tooth of *G. chessoia* is much wider at base and triangular
335 compared to that of *G. aegis* which is rectangular. Lateral teeth are sculptured in both
336 species, but the marks occur nearer to the base of the teeth in *G. aegis*. *G. chessoia* can
337 also be easily distinguished by the lack of sulphide deposits on shell and operculum, at

least from *G. aegis* found in Longqi Field, the only known habitat to date. Similarly, the operculum in *G. aegis* is much thicker than *G. chessoia* at all life stages.

Figure 8

***Gigantopelta aegis* sp. nov.** (Figs. 2-7)

Type material: Holotype. Shell diameter 37.61mm, 99% ethanol, Fig. 3D-F. Longqi vent field, Southwest Indian Ridge, 37°47.03'S 49°38.97'E ('Tiamat'), 2785m deep, RRS *James Cook* expedition JC67, ROV *Kiel 6000* Dive 142, 29.11.2011, leg. J. T. Copley (NHMUK 2015.XX). Paratypes. One dissected specimen, 99% ethanol (shell diameter 35.24mm, Fig. 4C-D; NHMUK 2015.XX); growth series of five specimens, 99% ethanol (NHMUK 2015.XX); growth series of five specimens, 99% ethanol OUMNH.ZC.2013.02.003); growth series of five specimens (SMNH Type Collection 8451). All paratypes above have the same collection data as holotype. Five specimens, 10% buffered formaldehyde (NHMUK 2015.XX): Longqi vent field, Southwest Indian Ridge, 37°47.03'S 49°38.96'E ('Tiamat' chimney), 2783m deep, RRS *James Cook* expedition JC67, ROV *Kiel 6000* Dive 140, 27.11.2011, leg. J. T. Copley (NHMUK 2015.XX).

Non-Type Materials Examined: Approximately 200 specimens, same collection data as the holotype.

Etymology: Aegis (Latin), the shield of Zeus and Athena. The specific name is an allusion of the thick and large sulphide-covered operculum to the mythical shield.

Description / Diagnosis:

Shell: Shell (Fig. 4B) globose, 3-4 whorls, trochiform to neritiform. Spire depressed. Aperture holostomous. Tightly coilded. Suture deep. Aperture very large, circular, body whorl to aperture length ratio approximately 1:0.65 (average of 100 specimens). Protoconch (Fig. 5B) 0.5 whorls, about 210 μm in length, sculpture unknown (surface layer of examined specimens affected by dissolution). Thick, orange to reddish sulphide layer covers periostracum. Periostracum dark olive with sulphides removed. Ostracum milky white. Ostracum thin, fragile without sulphide and periostracum. Periostracum slightly recurved at aperture. Columellar folds lacking. Callus extends extensively covering columellar region. Area around callus flattened (dark area in Fig. 3F). Shell smooth, lacking sculpture. Fine growth lines, subtle spiral cords present under sulphide layer. Maximum shell diameter 44.2mm.

Operculum: Operculum (Fig. 3E-F) corneous, thin, flaky near the fringe, multispiral, covered by thick sulphide layer except outermost whorl, same material as those covering shell. Juvenile operculum lacking sulphide layer. Moderately thick, opaque, with concave shape (Fig. 5B).

Radula: Radula (Fig. 6B) rhipidoglossate. Ribbon in adults approximately 0.5 mm wide and 4 mm long. Formula ~ 50 + 4 + 1 + 4 + ~ 50. Central, lateral teeth (Fig. 6D) with sharp cusps. Central tooth rectangular. Lateral teeth bear a protrusion near the base. Marginal teeth (Fig. 6F) elongate with truncate distal ending, dividing into ~ 20 denticles.

Soft parts (Fig. 7B): Foot muscular, large. Fully retractable. Pale white when alive. Small epipodial tentacles present, surrounding posterior 2/3 of operculum. Cephalic tentacles

thick, broad at base, tapering distally. Snout tapering, and thick. Esophageal gland huge (see Fig. 7B). Intestines forming a simple loop. Ctenidium bipectinate. Sexes separate. Gonads rather displaced towards the head-foot. Shell muscle large, horse-shoe shaped.

Distribution: Only known from Longqi vent field, Southwest Indian Ridge (approx. 37°47.03' S 49°38.96' E), around 2700m depth. Found mostly on areas of diffuse flow but also on chimneys of active black smokers.

Remarks: Similar to *Gigantopelta chessoia* n. sp., see *Comparative Remarks* above for comparison. The sulphide covering of the shell and that forming the thick coating on the operculum is remarkable. The coating only covers the outer side, and can be removed from operculum intact by inserting a blade in between. The adult shells are completely covered with sulphide. Sulphide deposition appears to start very early in development, and from the protoconch; as in young specimens (~5mm maximum diameter) sulphide is only present as a tablet on the apex and not covering the whole shell. The shell parameters are given in Table 2. The relationships between the six parameters measured were investigated, and they were linear across all life stages. Fig. 8B shows a scatterplot of shell diameter against shell height.

Systematic Position

Based on the current characterisation, the morphological information places the new genus in Peltospiridae. *Gigantopelta* does not exhibit sexual dimorphism which is

consistent with other peltospirids, whereas most neomphalid and melanodrymiid males have a left cephalic tentacle modified to become a penis. Also notable is the truncated and comb-like ends of marginal teeth (Fig. 6E-F), which in Neomphalina is only present in Peltospiridae and Melanodrymiidae, with members of the Neomphalidae having claw-like ends. Irregular net-like protoconch sculpture seen in *G. chessoia* n. sp. is similar to those of some peltospirid genera such as *Depressigyra* and *Pachydermia*.

Genetic Support

Genetic analysis of five haplotypes from each of the two new species of *Gigantopelta* and all COI sequences for neomphaline gastropods available in GenBank confirms the placement of the new genus within the Neomphalina. Fig. 9 shows the Bayesian consensus tree resulting from the analysis of the partitioned COI dataset using each codon position as a partition. As COI sequences alone cannot provide adequate resolution to clarify the familial relationships within this clade, we refrain from making any phylogenetic conclusions here. The purpose of the analysis is only to show that *Gigantopelta* forms a discrete lineage within Neomphalina. The phylogenetic relationship of *Gigantopelta* and other neomphalines needs to be resolved in a multi-gene phylogenetic study in the future.

Figure 9

Table 3 shows a maximum-likelihood distance matrix constructed from COI sequences of seven Peltospiridae genera (the 'scaly-foot gastropod' is assumed to be a separate genus), including *Gigantopelta*. All species used are type species of the genus, except *Nodopelta* where COI sequences of the type species *N. heminoda* McLean, 1989 were not available

so sequences for *N. subnoda* McLean, 1989 were used instead. Pairwise COI divergence between the six non-*Gigantopelta* genera averaged 22.30% (range 12.78%-28.49%), while their divergence from *Gigantopelta* averaged 22.80% (range 19.12%-28.14%), supporting the generic status of the latter.

Population Genetics

The genetic diversity of *Gigantopelta chessoia* sp. nov. and *G. aegis* sp. nov. are summarised in Table 4. From the COI sequence of 30 individual of each species sequenced, 370bp of overlapping fragment is used in the analyses here. From these, 10 haplotypes of *G. chessoia* and 12 haplotypes of *G. aegis* were found. In both species, there is one dominant haplotype shared by 15 individuals in *G. chessoia* and 18 by *G. aegis*. Three haplotypes, including the dominant haplotype, were shared by multiple individuals in *G. chessoia* and two in *G. aegis*, other haplotypes were recovered as singletons.

Table 4

Statistical parsimony networks of the data were constructed to visualise the relationship between the haplotypes of the two species, (Fig. 10). The non-dominant haplotypes differed from the dominant haplotypes by only four mutations at most, with the majority within one to two mutations. The COI networks of both species show a generally 'star-burst' pattern, which is indicative of recent rapid demographic expansion. This is supported by negative and significant Tajima's *D* for *G. aegis* and Fu's *Fs* values for both species (Table 4), which reflects an excess of rare polymorphisms in the sample and indicates either recent demographic expansion or evidence of a selective sweep (Fu,

1997). Furthermore, the mismatch analysis (Table 4) returned non-significant sums of squared deviation (SSD) and raggedness index, which signifies that both species do not deviate from the model of demographic expansion. The haplotype diversity was very high but the nucleotide diversity was low in both species, which may also be result of recent expansion.

Figure 10

The pairwise F_{ST} value shown in Table 5 is large and significant, revealing a very high level of genetic divergence between the two species ($F_{ST} = 0.8975$, $p < 0.001$). This strongly supports the morphological evidence which shows the two populations represent separate species, and indicates there is currently no genetic connectivity and interbreeding between the two species. This is also supported by the fact that there are no shared haplotypes between the two species, and the most similar haplotype between the two is separated by seven mutations (Fig. 10).

Discussion

The new genus *Gigantopelta* described herein is unusual among hydrothermal vent-endemic gastropods. The members attain an extremely large size for the clade Neomphalina, which are normally smaller than 15 mm in shell diameter (although *Neomphalus fretterae* McLean, 1981 reaches 30 mm). The only other known neomphaline to attain a similar size is the 'scaly-foot gastropod' from Indian Ocean vents (Van Dover *et al.,* 2001; Warén *et al.,* 2003; Nakamura *et al.,* 2012). The 'scaly-foot gastropod' is also the only other known gastropod species to house endosymbiotic

bacteria in an enlarged esophageal gland (Goffredi *et al.,* 2004). It is not clear whether this is a result of common ancestry or convergent evolution as the phylogenetic relationship between *Gigantopelta* and the 'scaly-foot gastropod' is currently unclear but is certainly of great interest for future studies.

Gigantopelta aegis is remarkable in the thick sulphide coating present on shell and operculum, though it is not clear whether the animal is responsible for controlling the deposit of sulphides. Future studies may reveal this to be an adaptation against predation or against hostile environmental conditions, in deep-sea hydrothermal vents where making the shell thicker with calcium carbonate is energetically costly because of the low pH of vent fluids. An example of such adaptation is seen in the 'scaly-foot gastropod' of the same family, which forms sclerites from sulphides and covers the shell with the same material (Yao *et al.,* 2010). Sulphides are abundant near hydrothermal vents and are perhaps the best available material to strengthen defensive structures in these extreme environments. However, as vents differ in their chemical and physical environment (Tivey, 2007) it is entirely possible that if *G. aegis* is found at another site in the future the specimens they may not have the sulphide overlay.

The population genetic analyses of the two *Gigantopelta* species show clearly that there is currently no gene flow between the two species in ESR and SWIR. However the two species are only 4.43% divergent in COI, and assuming the rate of the molecular clock is similar to the approximate rates in Vetigastropoda (substitution rate 1.2% per million years, Hellberg & Vacquier, 1999) this means the two species have been separated since approximately 1.85 million years ago (mya). Furthermore, a peltospirid substitution rate

506 can be calculated from the COI divergence of 11.2% in *Pachydermia laevis* Warén &
507 Bouchet, 1989 across the Easter Microplate (Matabos *et al*., 2011). The Easter Microplate
508 formed about 3.88 mya (Plouviez *et al*., 2013), the substitution rate of *P. laevis* COI is
509 thus 1.44% per million years. Estimating using this rate, the two *Gigantopelta* species
510 were separated approximately 1.54 mya. Both these estimates are very recent and
511 suggests before then gene flow existed at that time between the hydrothermal vents on the
512 two oceanic ridges, which was then cut off by a recent event. A similar scenario has been
513 reported with the yeti crab *Kiwa* for which two closely related species are also present on
514 ESR and SWIR for which the divergence was estimated at 1.5 mya with a 95%
515 confidence range of 0.6–2.6 mya (Roterman *et al*., 2013). Separation of the ESR and
516 SWIR *Kiwa* species was attributed to alterations in the intensity and latitude of the
517 Antarctic Circumpolar Current fronts during the Mid-Pleistocene Transition (0.65 to 1.2
518 mya) or recent reduction in number of vent fields between the ESR and SWIR vents
519 (Roterman *et al*., 2013). A similar close relationship is also suggested for two species of
520 eolepadid barnacles and suggests historic dispersal from west to east of these taxa driven
521 by the Antarctic Circumpolar current (Herrera *et al.*, 2015). The same events may have
522 caused the separation of the two *Gigantopelta* species.
523
524 The diversification estimate given is recent but is, very crude and subject to large error,
525 leaving much room for a future refinement. This also assumes species at hydrothermal
526 vents evolve at the same rate as the shallow water species, which remains to be evaluated.
527 In fact the rates are likely to be very different for vent species. Using five vent-endemic
528 invertebrate groups from the eastern Pacific including *Lepetodrilus* vent limpets
529 Vrijenhoek (2013) established a mean rate of 0.234% per million years for COI. If rates

for *Gigantopelta* is similar this will mean separation of the two species occurred approximately 9.47 million years ago. This mean rate is likely to be an underestimate of the true substitution rate however, as using an old vicariance event 28.5 mya to estimate COI substitution rates is problematic owing to saturation (Ho *et al*., 2011).

The ESR vents where *G. chessoia* occurs are 6,000 km away from the Longqi vent field where *G. aegis* occurs, and the evidence that the two species are very closely related and diverged only recently leads to the obvious question of the distribution of hydrothermal vents in between the ESR and Longqi vent fields and what communities inhabit them. A series of hydrothermal vents inferred to be active have been detected on SWIR near the Bouvet Triple Junction (Bach *et al.,* 2002), and if survey of these vents in the future uncovers another population of *Gigantopelta* it would certainly shed light on their evolutionary history.

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562
563

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715 **Figure Legends**

716

717 **Figure 1.** Map of deep-sea hydrothermal vent fields where *Gigantopelta chessoia* sp. nov.
718 and *G. aegis* sp. nov. are known to occur. This map was created using Esri ArcMap 10.1
719 (ESRI 2012) and General Bathymetric Chart of the Oceans (GEBCO) Grid Display
720 Ver.2.13 (BODC 2010). Data source: Bathymetry, GEBCO; continents data. ArcWorld
721 Supplement; oceanic ridges, United States Geologic Service (USGS). Abbreviations:
722 SWIR = South West Indian Ridge, CIR = Central Indian Ridge, SEIR = South East Indian
723 Ridge, A-AR = American-Antarctic Ridge, ESR = East Scotia Ridge, and MAR = Mid
724 Atlantic Ridge.

725

726 **Figure 2.** *In-situ* aggregations of the two new species of *Gigantopelta* gen. nov.: A, *G.*
727 *chessoia* at E2 segment, ESR; B, *G. aegis* at Longqi vent field, SWIR. Scale bars = 5cm.

728

729 **Figure 3.** *Gigantopelta chessoia* sp. nov., holotype (NHM 2013-XX): A, aperture view;
730 B, umbilical view; C. aperture view; scale bars = 1cm. *Gigantopelta aegis* sp. nov.,
731 holotype (NHM 2013-XX): A, aperture view; B, umbilical view; C, aperture view; scale
732 bars = 1cm.

733

734 **Figure 4.** *Gigantopelta chessoia* sp. nov., paratype shell (NHM 2013-XX): A, aperture
735 view; B, abaperture view; scale bars = 1cm. *Gigantopelta aegis* sp. nov., paratype shell
736 (NHM 2013-XX): A, aperture view; B, abaperture view; scale bars = 1cm.

737

738 **Figure 5.** Protoconchs: A, *Gigantopelta chessoia* sp. nov., scale bar = 100μm; B,
739 *Gigantopelta aegis* sp. nov., scale bar = 100μm. Juvenile operculum: C, *G. chessoia* sp.
740 nov., scale bar = 500μm; D, *G. aegis* sp. nov., scale bar = 500μm.
741
742 **Figure 6**. Radula. Overview: A, *Gigantopelta chessoia* sp. nov.; B. *Gigantopelta aegis* sp.
743 nov.; scale bars = 100μm. Central and lateral teeth close-up: C, *G. chessoia* sp. nov.; D, *G.*
744 *aegis* sp. nov.; scale bars = 20μm. Marginal teeth close-up: E, *G. chessoia* sp. nov.; F. *G.*
745 *aegis* sp. nov.; scale bars = 10μm.
746
747 **Figure 7.** Illustration of soft parts with the mantle partially removed: A, *Gigantopelta*
748 *chessoia* sp. nov.; scale bar = 1cm; B, *Gigantopelta aegis* sp. nov.; scale bar = 1cm.
749 Abbreviations: ct = ctnidium, dg = digestive gland, eg = esophageal gland, et = epipodial
750 tentacles, gd = gonad, pc = pericardium, ll = lateral lappet, o = operculum attachment, sn
751 = snout, t = cephalic tentacles.
752
753 **Figure 8.** Scatterplot of shell diameter vs shell height across a size range of 100
754 specimens: A, *Gigantopelta chessoia* sp. nov. (line of best fit formula: y = 0.9045x -
755 0.6278, $R^2 = 0.99$); B, *Gigantopelta aegis* sp. nov. (line of best fit forumula: y = 0.8823x
756 - 0.8362, $R^2 = 0.99$).
757
758 **Figure 9.** Consensus tree reconstructed from a 579bp fragment of COI gene using
759 Bayesian inference.
760

761 **Figure 10.** Haplotype parsimonious networks constructed from COI sequences of 30
762 specimens of: A, *Gigantopelta chessoia* sp. nov.; B, *Gigantopelta aegis* sp. nov. Open
763 circles are represented haplotypes, number inside the circles and sizes of the circles
764 corresponds to number of individuals sharing the haplotype. Closed circles are
765 hypothesised intermediate haplotypes that are not represented by sequences.

766 **Tables**

767

768 **Table 1.** List of taxa used in analyses with GenBank accession numbers.

Clade	Family	Taxa	Author of Taxa	COI
Neomphalina	Peltospiridae	*Nodopelta subnoda*	McLean, 1989	GU984280
Neomphalina	Peltospiridae	*Rhynchopelta concentrica*	McLean, 1989	GU984282
Neomphalina	Peltospiridae	*Depressigyra globulus*	Warén & Bouchet, 1989	DQ093519
Neomphalina	Peltospiridae	*Pachydermia laevis*	Warén & Bouchet, 1989	AB429222
Neomphalina	Peltospiridae	*Peltospira delicata*	McLean, 1989	FJ977764
Neomphalina	Peltospiridae	*Peltospira operculata*	McLean, 1989	GU984278
Neomphalina	Peltospiridae	*Peltospira smaragdina*	Warén & Bouchet, 2001	GQ160764
Neomphalina	Peltospiridae	'Scaly-Foot Gastropod'	Undescribed, COI from Nakamura *et al.* 2012	AB540646
Neomphalina	Peltospiridae	*Gigantopelta chessoia* sp. nov. Haplotype: gc01-gc05	This study	
Neomphalina	Peltospiridae	*Gigantopelta aegis* sp. nov. Haplotype: ga01-ga05	This study	
Neomphalina	Neomphalidae	*Cyathermia naticoides*	Warén & Bouchet, 1989	DQ093518
Neomphalina	Neomphalidae	*Lacunoides* sp. Kermadec	Undescribed, COI from Heβ *et al.* 2008	AB330999
Neomphalina	Melanodrymiidae	*Leptogyra inflata*	Warén & Bouchet, 1993	AB330998
Neomphalina	Melanodrymiidae	*Leptogyropsis inflata*	Hasegawa, 1997	AB365258
Neomphalina	Melanodrymiidae	*Melanodrymia aurantiaca*	Hickman, 1984	GQ160763
Cocculiniformia	Cocculinidae	*Cocculina messingi*	McLean & Harasewych, 1995	AY923910

769

770

771 **Table 2.** Shell parameters of *Gigantopelta chessoia* sp. nov. and *G. aegis* sp. nov. Range and proportion to shell diameter are calculated
772 from 100 specimens across a size range in each species.

773

Parameters (mm)	Shell			Aperture		Operculum
	Diameter	***Height***	***Width***	***Length***	***Height***	***Diameter***
			***Gigantopelta chessoia* sp. nov.**			
Holotype (NHM 2013-XX)	36.30	31.74	26.27	24.94	27.22	21.73
Paratype (NHM 2013-XX)	31.12	26.50	22.25	21.24	23.91	17.87
Range	4.21 ~ 45.47	3.30 ~ 40.92	3.50 ~ 29.77	2.92 ~ 30.46	3.24 ~ 31.53	2.24 ~ 26.62
Proportion to Shell Diameter	1	0.865	0.727	0.633	0.719	0.566
SD of Proportion	-	0.050	0.035	0.034	0.040	0.048
			***Gigantopelta aegis* sp. nov.**			
Holotype (NHM 2013-XX)	37.61	32.88	26.89	26.28	26.18	19.09
Paratype (NHM 2013-XX)	35.24	29.67	25.28	23.58	24.89	17.75
Range	4.87 ~ 44.83	3.42 ~ 39.21	3.33 ~ 32.63	2.60 ~ 31.05	3.20 ~ 30.66	1.92 ~ 23.79
Proportion to Shell Diameter	1	0.833	0.745	0.654	0.710	0.475
SD of Proportion	-	0.055	0.044	0.057	0.048	0.058

774

775

776 **Table 3.** Maximum-likelihood distance matrix of seven genera in Peltospiridae, including the two new species of *Gigantopelta* gen. nov.,
777 constructed from 579bp fragments of COI gene. Analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al.*
778 2004).

779

		1	2	3	4	5	6	7	8
1	*Peltospira operculata*								
2	*Depressigyra globulus*	23.36%							
3	*Nodopelta subnoda*	15.99%	18.85%						
4	*Pachydermia laevis*	18.88%	23.16%	12.84%					
5	*Rhynchopelta concentrica*	22.34%	23.84%	19.99%	23.83%				
6	'Scaly-Foot Gastropod'	25.72%	28.78%	25.21%	27.43%	26.99%			
7	*Gigantopelta chessoia* sp. nov.	21.83%	21.83%	19.20%	19.25%	27.09%	28.35%		
8	*Gigantopelta aegis* sp. nov.	21.86%	25.25%	21.44%	21.05%	29.00%	28.63%	4.43%	

780

781

782 **Table 4.** Genetic diversity in COI (370bp fragment) of the two new species of *Gigantopelta* gen. nov. Shown for each species are: sample
783 size (*n*), number of haplotypes, number of polymorphic loci, haplotype diversity ($h \pm$ SD), nucleotide diversity ($\pi \pm$ SD), Tajima's *D* value,
784 Fu's *Fs* value, sum of square deviations of the mismatch distribution (SSD) and raggedness index from the mismatch analyses.

Species	*n*	Haplotypes	Polymorphic Loci	$h \pm$ SD	$\pi \pm$ SD	Tajima's *D*	Fu's *Fs*	SSD	Raggedness
Gigantopelta chessoia sp. nov.	30	10	9	0.7287 ± 0.0780	0.0037 ± 0.0026	-1.2271	-5.0511 **	0.0060	0.0147
Gigantopelta aegis sp. nov.	30	12	12	0.6460 ± 0.1014	0.0027 ± 0.0021	-2.2056 **	-10.6953 ***	0.0396	0.1356

* p < .05; ** p < .01; *** p < .001.

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788 **Table 5.** F-statistics based on pairwise comparisons of COI haplotype frequencies of the two new species of *Gigantopelta* gen. nov.

789 constructed from 370bp fragments of COI gene of 30 individuals from each species.

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	Gigantopelta chessoia sp. nov.	*Gigantopelta aegis* sp. nov.
	Pairwise FST	
Gigantopelta chessoia sp. nov.	0.0000	-
Gigantopelta aegis sp. nov.	0.8975 ***	0.0000

Note. FST = Fixation Index; Number of permutaions: 10000. * p < .05; ** p < .01; *** p < .001.