DESCRIPTION OF TWO NEW SPECIES OF INDO-PACIFIC THYLACOPLETHUS AND A NEW RECORD OF THOMPSONIA JAPONICA (RHIZOCEPHALA: THOMPSONIIDAE) FROM HERMIT, PORCELAIN, AND MUD CRABS (DECAPODA) BASED ON MORPHOLOGICAL AND MOLECULAR DATA

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ABSTRACT
Rhizocephalans of the order Akentrogonida are parasitic barnacles that infest decapods, stomatopods, peracarids, and other cirripedes. Within this order, Thompsoniidae is found in decapods and stomatopods, and is a family comprised of species characterized by having multiple globular reproductive bodies (externae) erupting from the host’s extremities and abdominal surface, and connected internally by a root-like system. Two of the four genera currently accepted within Thompsoniidae, Thompsonia and Thylacoplethus, contain perhaps the most morphologically derived species within Akentrogonida. Herein we describe two new species of Thylacoplethus, T. porcellanus and T. umanguvatus, from the porcellanid and hermit crabs Petrolisthes scabriculus (Dana, 1852) and Calcinus morgani Rahayu and Forest, 1999, respectively. We also redescribe Thompsonia japonica Häfele, 1911 from a xanthid brachyuran crab based on morphological characters and DNA sequences of the nuclear 18S rDNA gene. The new species from the porcellanid crab comprises the first record of an akentrogonid rhizocephalan parasitizing a species of Porcellanidae. We reconstruct a molecular phylogeny of Akentrogonida using new and published DNA sequences, and we discuss our findings within an evolutionary context of host speciation. All species in the four thompsoniid genera are briefly reviewed, and a replacement name is proposed for the preoccupied Pottsia Høeg and Lützen, 1993.

KEY WORDS: Anomura, Brachyura, Cirripedia, Porcellanidae, Pottsia, Rhizocephala
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INTRODUCTION
Rhizocephala (Thecostraca: Cirripedia) is a group of parasitic barnacles containing more than 260 species highly specialized to infest mostly decapod crustaceans (Caridea, Brachyura, and Anomura) but also peracarids (Isopoda), stomatopods, and other cirripedes (Høeg, 1995; Walker, 2001; Boyko and Williams, 2009; Pérez-Losada et al., 2009; Glenner et al., 2010). These parasites are interesting from an evolutionary perspective, as their extreme adaptation to parasitic life has resulted in the adults losing almost all morphological characters normally found in Crustacea. In fact, their affinities with other cirripedes were discovered through study of their larval stages (Thompson, 1836; Delage, 1884; Martin et al., 2014).

Rhizocephala is divided into two orders, Kentrogonida (three families) and Akentrogonida (six families), based on the presence or absence in the reproductive cycle of a post-settlement stage (kentrogon) responsible for infestation of the host (Høeg, 1995; Walker, 2001; Glenner et al., 2010; Yoshida et al., 2011). Glenner et al. (2010) provided molecular evidence that akentrogonids exhibit a derived loss of the kentrogonid stage because this group is nested within a paraphyletic Kentrogonida. Kentrogonids are only known from decapod hosts whereas akentrogonids have a wider diversity of hosts, and exhibit variation in aspects of their life history (Glenner et al., 2010). Coloniality has evolved independently in both orders of rhizocephalans, e.g., Kentrogonida: Peltogasterella spp.; Akentrogonida: Thompsonia spp. (Høeg and Lützen, 1995; McDermott et al., 2010).

Rhizocephalans have a direct life cycle (only one host) and produce larvae of separate sexes (genetically or environmentally determined) (Yamaguchi et al., 2014). Female cyprid larvae attach to an un sclerotized cuticular surface of the host, and inject an infective stage that forms a nutrient-absorbing system of rootlets called the interna. After a period of development, the female adult-stage forms an external reproductive body called the externa. Male cyprids are attracted to the virgin female externa on the infected host, and fertilize it through invasion of the mantle aperture by a trichogon (kentrogonids), or via implantation of male material through the cyprid antennule (akentrogonids) (Høeg, 1995; Walker, 2001). Rhizocephalans eventually come to

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control aspects of their host’s biology (e.g., physiology and behavior) and can modify their morphology (feminization) (Ritchie and Høeg, 1981; Høeg, 1995). Host specificity seems to vary considerably (see review in Boyko and Williams, 2009), with some species, e.g., *Sacculina carcini* Thompson, 1836, being known from multiple host species (Thresher et al., 2000).

Species of akentrogonid barnacles within Thompsoniidae belonging to *Diplothylacus* Høeg and Lützen, 1993, *Pottsia* Høeg and Lützen, 1993, *Thompsonia* Kossmann, 1872, and *Thylacoplethus* Coutière, 1902, are characterized by globular externae erupting colonially on the body surfaces of decapods (Fig. 1) such as shrimp (Caridea), true crabs (Brachyura), hermit crabs, and squat lobsters (Anomura: Paguroidea, Chirostylidea, and Galatheoidea), as well as more distantly related mantis shrimps (Stomatopoda) (Høeg and Lützen, 1993; Walker, 2001; Boyko and Williams, 2009). *Diplothylacus* and *Pottsia* contain two species each, *Thompsonia* contains five species, while *Thylacoplethus* includes 11 species (Boxshall and Boyko, 2014) plus two new species described herein (see Table 3 below).

Note that *Pottsia* Høeg and Lützen is a homonym of *Pottsia* Chatton and Lwoff, 1927, and is here replaced by *Jensia* Boyko and Williams, nom. nov. in the Appendix; *Jensia* is used as the correct name for the genus in the remainder of this paper. Thompsoniid species are among the most derived of akentrogonids (Glenner and Hebsgaard, 2006; Pérez-Losada et al., 2009; this study); they are most noteworthy for a remarkable simplification of morphology and loss of characters.

Examination of porcellanids recently collected in Australia revealed that a male *Petrolisthes scabriculus* (Dana, 1852) was parasitized by a thompsoniid rhizocephalan (Fig. 1B). Although several species of kentrogonids in Lernaeodiscidae Müller, 1862, have been described from porcelain crab hosts (Boyko and Harvey, 2000), this is the first
record of any akentrogonid parasitizing a porcellanid crab. In order to determine whether this parasite belongs to Thylacoplethus or Thompsonia we did the following: 1) characterized the morphology of the externae and compared it to that of other species in these genera; and 2) obtained a DNA sequence of a fragment of the nuclear 18S rDNA gene, compared it to other sequences available in GenBank, and reconstructed the phylogeny of Akentrogonida, which was shown by Glenner et al. (2010) to be monophyletic. In order to have a reference value of 18S rDNA sequence divergence between crustacean sister taxa sharing a relatively recent common ancestor, we estimated the 18S rDNA distance between Caribbean and Pacific individuals of the amphipod Porcellanidae Petrolisthes armatus (Gibbes, 1850), and of the gerninate P. glasselli Haig, 1957 (eastern Pacific) and P. rosariensis Werding, 1982 (western Atlantic). Populations of P. armatus on both American coasts were probably separated 2.8 million years ago when emergence of the Central American Isthmus was completed (Lessios, 2008). The gerninate pair seems to have diverged before the isthmus closed completely (Hiller et al., 2006).

Morphological and molecular information indicates that the species infesting P. scabriculus is a new species of Thylacoplethus. We describe this new species and discuss its origin within a phylogenetic context of host speciation. Additionally, we report on the morphology and 18S rDNA sequence of akentrogonids from a xanthid crab collected in Bali, Indonesia, which are morphologically indistinguishable from both Thompsonia littoralis Lützen and Jespersen, 1990 and Thompsonia japonica Häfele, 1911, and discuss the problems in assigning them to species. Finally, we report on a new species of Thylacoplethus from hermit crabs of the genus Calcinus Dana, 1851, from Bali, Indonesia, from which we were unable to obtain DNA sequences, but which is morphologically distinct from all other species in the genus.

MATERIALS AND METHODS

Akentrogonid externae were taken from the dorsal and ventral surfaces of the walking legs and inner surface of the pleon of a P. scabriculus, the pereiopods of a Lophozymus dodone (Herbst, 1801), and the pereiopods of 11 Calcinus morgani Rahayu and Forest, 1999. Externae were morphologically characterized, and some were used for molecular analyses. None of the host specimens were collected with the original intent of studying the rhizocephalans, and therefore, none were fixed in a manner conducive to histological examination. Host names have been updated using WoRMS (World Register of Marine Species) to reflect current classifications. Taxonomic authorities and dates for hosts and parasites are provided in Tables 1 and 3.

Specimens were borrowed from or are deposited in the following institutions: the Florida Museum of Natural History (UF), Gainesville, FL, USA; the Smithsonian National Museum of Natural History (USNM), Department of Invertebrate Zoology (Museum Support Center), Suitland, MD, USA; and the Justus-Liebig University (JLU), Department of Animal Ecology and Systematics, Gießen, Germany. As these akentrogonids are colonials organisms, we have designated all externae from each type host specimen as representing a single holotype. Size of host is given as carapace length (CL) × width (CW) for xanthid and porcellanid hosts, or shield length (SL) for hermit crab hosts. All means are reported with ± standard deviation.

Morphological Analysis

Externae of all three species were examined using light and scanning electron microscopy (SEM). Line drawings of externae were made with an Olympus SZX dissecting microscope with a drawing tube attachment. Original drawings were scanned and traced in Adobe Illustrator. Measurements of externae were made from digital images with Image J. Externae were prepared for SEM by dehydrating in an ascending ethanol series (70% to 95% EtOH), followed by four changes of 100% EtOH. Specimens were critical point dried (Sandri 795 Critical Point Dryer), mounted on aluminum stubs, coated with gold (EMS-550 sputter coater), and viewed with a FEI Quanta 250 SEM.

Molecular Analysis

DNA Extraction, Amplification and Sequencing.—DNA was isolated from externae taken from the three host species, from two specimens of P. armatus, and one specimen each of P. glasselli and P. rosariensis (see Table 1), using the QIAGEN DNeasy® Blood & Tissue Kit following the animal tissue protocol.

An approximately 470-bp fragment of the nuclear 18S rDNA gene was amplified through PCR (polymerase chain reaction; Saiki et al., 1988) using the primers 18SRhizoF2 (5′-GCCAGTAATGATATGCTTGTC-3′) and 18SRhizoR3 (5′-CTGCTGCTCTTTAGTC-3′). At this point we want to report that we started testing PCR amplifications using different combinations of the primers used by Pérez-Losada et al. (2009) to obtain approximately 2000-bp sequences of different Thococrana. Failure to obtain PCR products using these primers, probably due to degraded DNA, prompted us to design a series of new, specific primers to amplify fragments between 400 bp and 700 bp using the software Primer Premier 6.12 (PREMIER Biosoft). PCR amplifications (25 μl total volume) were performed using the Platinum® Taq DNA Polymerase Kit (Life Technologies) and 8 mM dNTPs (Promega) following the manufacturer’s protocol, using an annealing temperature of 50°C, and 1.3 μl of DNA. PCR products were purified by running them in a 2% TAE electrophoresis, excising the desired band, and incubating it with 1.5 μl GELase™ (Epicentre) at 70°C for 25 min and at 45°C for 3 h. Cycle sequencing reactions were performed in both sequencing directions using the BigDye Terminator Kit (Applied Biosystems) following the manufacturer’s protocol, then cleaned using Illustra™ Sephadex® G-50 (GE Healthcare Life Sciences) and the MultiScreen® Assay System 96-well filtration plate (EMD Millipore), and finally electrophoresed in an ABI-PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Sequence Alignment and Phylogenetic Analysis

The BioEdit Sequence Alignment Editor (Hall, 1999) was used for sequence edition. Alignment of sequences obtained in this study, and of those retrieved from GenBank (Table 1) was performed using MAFFT v7.149b (Katoh and Standley, 2013). The Akaike Information Criterion (AIC; Akaike, 1974), implemented in the program jModeltest2 (Guindon and Gascuel, 2003; Darriba et al., 2012), was used to select the model of nucleotide substitution that best fits the data. The model selected was GTR + I + G, with I = 0.297 and G = 0.464. The estimated parameter values were incorporated as priors into a Bayesian analysis performed with MrBayes v3.2.2. (Ronquist et al., 2012). Chains were run for the generations needed for the two parallel runs to converge into stationary distribution (value of the standard deviation of split frequencies < 0.01). The number of samples was set to 10% of the number of generations. Burnin was set to 25% of the samples. Kimura 2-parameter distances (K2P; Kimura, 1980) were estimated between all possible pairs of sequences using MEGA v6.06 (Tamura et al., 2013) setting the Gamma parameter (alpha) according to the jModeltest2 results.

Table 1 lists the GenBank accession-number codes of the sequences used in the present molecular analyses.

RESULTS

The alignment of 10 partial sequences of the 18S rDNA gene from akentrogonid Rhizocephala, including the new Petrolisthes parasite and the xanthid parasite, and from one kentrogonid, Polycus polygenea (Lützen and Takahashi, 1997) used as out-group, was 486 bp long. Sequences from the specimens parasitizing Calcinus failed to amplify and were not included in the analysis. The phylogenetic reconstruction obtained through Bayesian inference is shown in Fig. 2. All sequences representing mem-
Table 1. List of akentrogonid rhizocephalans included in the molecular analysis, their host species, sampling localities, museum catalog numbers and GenBank (GB) accession numbers of partial 18S rDNA sequences with respective references. A ken trotgonid (Polysaccus polygenea) was used as an outgroup to root the 18S rDNA molecular tree. Two Petrolisthes armatus from the Atlantic and Pacific coasts of Panama, and one specimen each of P. glasselli (East Pacific) and P. rosariensis (West Atlantic) were sequenced for this gene to estimate sequence divergence in transisthmian and geminate species (see text). UF, Florida Museum of Natural History, Gainesville, FL, USA; USNM, Smithsonian National Museum of Natural History (Museum Support Center), Suitland, MD, USA; JLU, Justus-Liebig University Giessen, Giessen, Germany; N/A, not applicable.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Collection locality</th>
<th>GB accession number with reference/museum catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thylacoplethus porcellanus n. sp.</td>
<td><em>Petrolisthes scabriculus</em> (Dana, 1852) (Porcellanidae)</td>
<td>Heron Island, QLD, Australia</td>
<td>KP739771 (this study)/UF34625</td>
</tr>
<tr>
<td>Thylacoplethus magellani Høeg and Lützen, 1993</td>
<td><em>Pagurus forcps</em> H. Milne Edwards, 1836 (Paguridae)</td>
<td>Beagle Island, Argentina</td>
<td>FJ751889 (Pérez-Losada et al., 2009)</td>
</tr>
<tr>
<td>Thompsonia japonica Høeg and Lützen, 1993</td>
<td><em>Lophozozymus dodone</em> (Herbst, 1801) (Xanthidae)</td>
<td>Sanur, Bali, Indonesia</td>
<td>KP739770 (this study)/USNM1268865, 1268866</td>
</tr>
<tr>
<td>Thompsonia littoralis Lützen and Jespersen, 1990</td>
<td><em>Leptodius exaratus</em> (H. Milne Edwards, 1834) (Xanthidae)</td>
<td>Labrador Park Beach, Singapore</td>
<td>DQ826573 (Glenner and Høeg, 2006)</td>
</tr>
<tr>
<td>Potsia serenei Lützen and Du, 1999</td>
<td><em>Squilla sp.</em> (Squillidae)</td>
<td>Vietnam</td>
<td>DQ826567 (Glenner and Høeg, 2006)</td>
</tr>
<tr>
<td>Diplothylacus sinensis (Keppen, 1877)</td>
<td><em>Portunus pelagicus</em> (Linnaeus, 1758) (Portunidae)</td>
<td>Singapore</td>
<td>DQ826568 (Glenner and Høeg, 2006)</td>
</tr>
<tr>
<td>Polysaccus japonicus Høeg and Lützen, 1993</td>
<td><em>Nihonotrypaea japonica</em> (Ortmann, 1891) (Callianassidae)</td>
<td>Tomioka, Japan</td>
<td>DQ826565 (Glenner and Høeg, 2006)</td>
</tr>
<tr>
<td>Clistosaccus paguri Lilljeborg, 1861</td>
<td>Hermit crabs (Paguridae)</td>
<td>Kristineberg, Sweden</td>
<td>GU190697 (Glenner et al., 2010)</td>
</tr>
<tr>
<td>Sylton hippolytes M. Sars, 1870</td>
<td><em>Pandalus</em> sp. (Pandalidae)</td>
<td>Vancouver Island, Canada</td>
<td>DQ826564 (Glenner and Høeg, 2006)</td>
</tr>
<tr>
<td>Boschmaella japonica Deichmann and Høeg, 1990</td>
<td><em>Chthamalus challengeri</em> Hoek, 1883 (Chthamalidae)</td>
<td>Jōgashima, Kanagawa Prefecture, Japan</td>
<td>AY265369 (Glenner et al., 2003)</td>
</tr>
<tr>
<td>Polyascus polygenea (Lützen and Takahashi, 1997)</td>
<td><em>Hemigrapsus sanguineus</em> (De Haan, 1835) (Varunidae)</td>
<td>Kyushu, Japan</td>
<td>AY265362 (Glenner et al., 2003)</td>
</tr>
<tr>
<td>Petrolisthes armatus (Gibbes, 1850)</td>
<td>N/A</td>
<td>María Chiquita, Panama, Caribbean</td>
<td>KP739767 (this study)/JLU1001</td>
</tr>
<tr>
<td>Petrolisthes glasselli Haig, 1957</td>
<td>N/A</td>
<td>Isla Nao, Panama, East Pacific</td>
<td>KP739766 (this study)/JLU1000</td>
</tr>
<tr>
<td>Petrolisthes rosariensis Werding, 1978</td>
<td>N/A</td>
<td>Islas Secas, Panama, East Pacific</td>
<td>KP739768 (this study)/JLU1003</td>
</tr>
</tbody>
</table>
bers of Thomposiidae [Diplothyacius sinensis (Keppen, 1877), Jenisia serenei (Høeg and Lützen, 1993), Thompsonia littoralis, Thylacoplethus magellani Høeg and Lützen, 1993, and the new sequences from Thompsonia japonica and Thylacoplethus porcellanus n. sp.] nest monophyletically with a relatively high posterior probability (94%), and show Boschmaelia japonica Høeg, Kapel, Thor, and Webster, 1990 (Cthamalophiliidae) as sister taxon to Thomposiidae. Clistosaccus paguri Lilljeborg, 1861 and Sylon hippolytes M. Sars, 1870 (Clistosaccidae) are a monophyletic group with a high posterior probability value (100%), and join Polysaccus japonicus Høeg and Lützen, 1993 in forming a polytomy with the thompsoniid clade. Thylacoplethus and Thompsonia are recovered as sister taxa, sufficiently separated to be considered separate genera, as suggested by Høeg and Lützen (1993), although convincing morphological synapomorphies are still lacking (see below). Jenisia is the sister taxon to Thompsonia + Thylacoplethus. The new species parasitizing the porcellanid crab is closely related to Thylacoplethus magellani, and the species found on the xanthid crab (Thompsonia japonica) is close to, but distinct from, Thompsonia littoralis.

The K2P distances estimated between all possible pairs of rhizocephalan 18S rDNA sequences using the Kimura two-parameter (K2P) model are shown in Table 2. The mean distance of pairwise comparisons within the clade containing Tylacoplethus and Thompsonia is 15.1%, with the lowest distances corresponding to the Tylacoplethus magellani-Thylacoplethus porcellanus pair (7.7%) and the Thompsonia littoralis-Thompsonia japonica pair (12.1%). The mean sequence divergence between the two genera is 17.7%. No divergence (0%) in the sequence fragment of the 18S rDNA gene was found between Caribbean and Pacific individuals of the porcellanid Petrolithes armatus. The distance between the gennmates P. glasselli and P. rosariensis is 7.2%. These values are reference indicators that support the conclusion that the species here described are different from their putative sister species.

Data are available from the Dryad (http://doi.org/10.5061/dryad.67s7p8) and TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S16937) digital repositories.

### Systematics

**Rhizocephala Müller, 1862**

**Akenkrogonida Häfele, 1911**

**Thomposiidae Høeg and Rybakov, 1992**

**Thompsonia Kossmann, 1872**

**Thompsonia japonica Häfele, 1911**

(Figs. 1A, 4A, B)

Material Examined.—130 externae on a female Lophoziomythus dodone (CL = 10.7 mm, CW = 16.1 mm), shallow intertidal, coral rubble, 8°42′5.60″S, 115°15′56.82″E, Sanur, Bali, Indonesia, 6 August 1997, J. D. Williams coll. (USNM1268865 in alcohol, USNM1268866 on SEM stub).

Redescription.—Externae ovoid, immature, 1.2-1.6 mm in length (1.36 ± 0.13 mm, n = 10), up to 0.8 mm in diameter (0.76 ± 0.07 mm, n = 10), set on short stalk (Figs. 1A, 4A, B). Stalk with single annulus, positioned at base of stalk (Fig. 4A, B). Mantle thin and transparent, filled with ovarian tissue. Mantle aperture lacking.

In total, 130 externae on host (host damaged with left pereiopods 2 and 3 detached); 39 externae on ventral abdominal surface, 1-18 externae on pereiopods 1-5 (mostly on merus but a few on basis or ischiu).

Remarks.—Thompsonia and Thylacoplethus are purportedly distinguished by the following three characters: stalk annulus number (one in Thompsonia, two in Thylacoplethus), mantle pore opening (absent in mature Thompsonia externae, present in Thylacoplethus), and spermatogenic bodies within the mantle (present in Thompsonia, absent in Thylacoplethus) (Høeg and Lützen, 1993). However, none of these characters is consistent across species within the two genera. Some species of Thylacoplethus have a mantle pore in mature externae, no spermatogenic bodies, but only one annulus, e.g., Thylacoplethus piloidae Lützen and Jespersen, 1990, Thylacoplethus orientalis Høeg and Lützen, 1993; however, another species, Thylacoplethus isaeva Rybakov and Shukalyuk, 2004, has two annuli, a mantle pore (only in the most mature specimens), and spermatogenic bodies; only one of these characters (the number of annuli) has been documented for all five species of Thompsonia, but only Thompsonia littoralis has been well described.

The present specimens on the xanthid host are morphologically indistinguishable from Thompsonia globosa Kossmann, 1872, Thompsonia japonica, and Thompsonia lit-
<table>
<thead>
<tr>
<th>Parasitic species</th>
<th>Host</th>
<th>Parasite locality (Host’s geographic range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diplothylacus sinensis</strong> (Keppen, 1877)</td>
<td><em>Portunus pelagicus</em> (Linnaeus, 1758) and possibly <em>P. sanguinolentus</em> (Herbst, 1783) (Brachyura: Portunoidea: Portunidae)</td>
<td>China, Singapore, West Malaysia, Sarawak, Thailand (Indo-Pacific)</td>
</tr>
<tr>
<td></td>
<td><em>Thalamita prymna</em> (Herbst, 1783) and <em>T. admete</em> (Herbst, 1803) (Brachyura: Portunoidea: Portunidae)</td>
<td>Northeast Australia (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Jensia serenei</strong> (Lützen and Du, 1999)</td>
<td><em>Oratosquilla gravierei</em> (Manning, 1978) (Stomatopoda: Squilloidea: Squillidae)</td>
<td>Vietnam (Vietnam to Philippines, New Caledonia and Australia)</td>
</tr>
<tr>
<td><strong>Thompsonia affinis</strong> Krüger, 1912</td>
<td><em>Uroptychus</em> sp. and <em>U. tomentosus</em> Baba, 1974 (Anomura: Chirostyloidea: Chirostylidae)</td>
<td>Japan and New Zealand (specific ID and range unknown for Japan specimen; <em>U. tomentosus</em> – New Zealand)</td>
</tr>
<tr>
<td><strong>Thompsonia chuni</strong> Häfele, 1911</td>
<td><em>Parapagurus</em> sp. (Anomura: Paguroidea: Parapaguridae)</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Thompsonia globosa</strong> Kossmann, 1872</td>
<td><em>Lybia tessellata</em> (Lateville, 1812) (Brachyura: Xanthoidea: Xanthidae)</td>
<td>Philippines (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Thompsonia japonica</strong> Häfele, 1911</td>
<td><em>Pilumnus dolleini</em> Balss, 1933; <em>Actumnus squamosus</em> (De Haan, 1835) (Brachyura: Pilumnoidea: Pilumnidae); <em>Lophozoymus dodone</em> (Herbst, 1801) (Brachyura: Xanthoidea: Xanthidae)</td>
<td>Japan, Indonesia (<em>P. dolleini</em> – Japan; <em>A. squamosus</em> – Indonesia; <em>L. dodone</em> – Indonesia)</td>
</tr>
<tr>
<td><strong>Thompsonia littoralis</strong> Lützen and Jespersen, 1990</td>
<td><em>Pilidius granulatus</em> Stimpson, 1858; <em>Cyclodiopsis granulosus</em> (De Man, 1888); <em>Chlorodiella cytherea</em> (Dana, 1852) (Brachyura: Xanthoidea: Xanthidae)</td>
<td>Philippines, Indonesia (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Thylacoplethus afric anus</strong> Høeg and Lützen, 1993</td>
<td><em>Gaillardiella rupeppeli</em> (Krauss, 1843) (Brachyura: Xanthoidea: Xanthidae)</td>
<td>South Africa (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Thylacoplethus cubensis</strong> (Reinhard and Stewart, 1956)</td>
<td><em>Munida stimpsoni</em> A. Milne Edwards, 1880 (Anomura: Galatheoidea: Munididae)</td>
<td>Cuba (Caribbean)</td>
</tr>
<tr>
<td><strong>Thylacoplethus edwardsi</strong> Coutière, 1902</td>
<td><em>Alpheus malleodigitus</em> (Bate, 1888), <em>A. edwardsii</em> (Audouin, 1826), <em>A. strenuus</em> Dana, 1852, “A. sulcatus” Kingsley, 1878 (Caridea: Alpheoidea: Alphidae); <em>Lysmata kempi</em> Chace, 1997 (Caridea: Alpheoidea: Hippolytidae)</td>
<td>Fiji, Australia, Taiwan (Indo-Pacific; <em>A. sulcatus</em> is an eastern Pacific species and this record is from an Indo-Pacific member of the <em>sulcatus</em> species group)</td>
</tr>
<tr>
<td><strong>Thylacoplethus isaevae</strong> Rybakov and Shukalyuk, 2004</td>
<td><em>Pagurus trigonocheirus</em> (Stimpson, 1858) (Anomura: Paguroidea: Paguridae)</td>
<td>Sakhalin (Arctic coasts of Alaska and Siberia to Bering Sea, Aleutian Islands, and Kamchatka)</td>
</tr>
<tr>
<td><strong>Thylacoplethus magellani</strong> Høeg and Lützen, 1993</td>
<td><em>Pagurus forcipes</em> H. Milne Edwards, 1836 (Anomura: Paguroidea: Paguridae)</td>
<td>Chile (Chile, Argentina)</td>
</tr>
<tr>
<td><strong>Thylacoplethus minius</strong> Lützen and Nagasawa, 1998</td>
<td><em>Synalpheus stimpsonii</em> (De Man, 1888) (Caridea: Alpheoidea: Alphidae)</td>
<td>Australia (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Thylacoplethus novaeanzelandiae</strong> Lützen, Glenner and Lörz, 2009</td>
<td><em>Uroptychodes epigaster</em> Baba, 2004 (Anomura: Chirostyloidea: Chirostylidae)</td>
<td>New Zealand (New Caledonia, New Zealand)</td>
</tr>
<tr>
<td><strong>Thylacoplethus orientalis</strong> Høeg and Lützen, 1993</td>
<td><em>Dardanus impressus</em> (De Haan, 1849); <em>D. arrosor</em> (Herbst, 1796) (Anomura: Paguroidea: Diogenidae)</td>
<td>Japan (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Thylacoplethus piloidae</strong> (Lützen and Jaspersen, 1990)</td>
<td><em>Pilidius nigrocrinitus</em> Stimpson, 1859 (Brachyura: Xanthoidea: Xanthidae)</td>
<td>Singapore (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Thylacoplethus porcellanus</strong> n. sp.</td>
<td><em>Petrolisthes scabriculus</em> (Dana, 1852) (Anomura: Galatheoidea: Porcellanidae)</td>
<td>Australia (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Thylacoplethus reinhardi</strong> (Lützen, 1992)</td>
<td><em>Discorsopagurus schmitti</em> Stevens, 1925; possibly <em>Pagurus capilatus</em> (Benedict, 1892) (Anomura: Paguroidea: Paguridae)</td>
<td>Washington, USA (northeast Pacific, Japan – <em>D. schmitti</em> only)</td>
</tr>
<tr>
<td><strong>Thylacoplethus squillae</strong> Høeg and Lützen, 1993</td>
<td><em>Orotopsis oratoria</em> (De Haan, 1844), <em>Harpiosquilla harpax</em> (De Haan, 1844) (Stomatopoda: Squilloidea: Squillidae)</td>
<td>Japan, Vietnam (Indo-Pacific)</td>
</tr>
<tr>
<td><strong>Thylacoplethus umanguvatus</strong> n. sp.</td>
<td><em>Calcinus morgani</em> Rahayu and Forest, 1999 (Anomura: Paguroidea: Diogenidae)</td>
<td>Bali, Indonesia (Indo-Pacific)</td>
</tr>
</tbody>
</table>
However, *Thompsonia globosa* is extremely poorly described and cannot be convincingly identified without collection of topotypic material (see Table 3). According to Lützen and Jespersen (1990), *Thompsonia japonica* and *Thompsonia littoralis* can only be distinguished based on the carapace length of the cyprid larvae. No molecular data exists for *Thompsonia japonica*, but an 18S rDNA sequence exists for *Thompsonia littoralis* (Glenner and Hebsgaard, 2006) that was derived from either paratypic or topotypic material (which one is not clear). Based on molecular data, our specimens are close to, but distinct from *Thompsonia littoralis* (Table 2). Because there are no characters in our specimens to differentiate them from *Thompsonia littoralis*, we have conservatively identified them with the latter species. *Thompsonia japonica* is also known from two other xanthid crabs (see Table 3). The single record from a hermit crab (*Pagurus striatus* Latreille, 1802 = *Dardanus arrosor* Herbst, 1796)) given by Krüger (1912) is considered by Høeg and Lützen (1993) to represent *Thylacoplethus orientalis*.

*Thylacoplethus* Coutière, 1902

**Thylacoplethus porcellanus** n. sp.

(Figs. 1B, 3A, 4C, D)

Material Examined.—13 externae (UF34625, holotype) on one male *Petrolisthes scabriculus* (UF25638; CL = 2.85 mm, CW = 2.85 mm), in rubble, 23°27′15.48″S, 151°52′1.56″E, north Wistari, Heron Island, Queensland, Australia, 23 November 2009, L. Avery, K. Schnabel, N. Bruce coll.

Description.—Externae ovoid, mature, 0.7 to 1.5 mm in length (1.0 ± 0.3 mm, n = 13), up to 0.6 mm in diameter, set on short stalk (Figs. 1B, 3A, 4C). Stalk with single, conspicuous annulus approximately at its mid-length (Figs. 3A, 4D). Mantle thin and transparent, filled with eggs. Mantle aperture lacking.

In total, 17 externae on host; 6 on ventral pleonal surface, not more than 2 per pleomere, symmetrical around medial line of each segment, not found on tail fan or sixth pleomere, 11 on pereiopods (mostly on merus but a few on basis or ischium) (Fig. 1B).

Etymology.—The species is named based on the host, a crab of Porcellanidae, and is a noun in apposition; the gender is masculine.

**Fig. 3.** A, *Thylacoplethus porcellanus* n. sp. (holotype, UF34625), lateral view of individual externae of holotype, two externae with host fragment remaining at base of stalks; B, *Thylacoplethus umanguvatus* n. sp. (holotype, USNM1268852), lateral view of individual externae of holotype; developing cyprids within externa shown. Scale bar = 0.5 mm.
Fig. 4. A, B, Thompsonia japonica (USNM1268866); A, five externae in situ on fifth leg of host (arrow indicates single cuticular annulus at base of stalk); B, apical view of externa showing lack of pore. C, D, Thylacoplethus porcellanus n. sp. (holotype, UF34625); C, whole externae, lateral view; D, stalk of externa, showing single cuticular annulus (arrowhead) and thalli belonging to an unidentified mesomycetozoean (horizontal arrow). E-H, Thylacoplethus umanguvatus n. sp. (paratypes, USNM1268863, 1268864), scanning electron micrographs; E, two externae in situ on posterior pereiopod of host; several scars of externae are also shown (black arrowhead shows scar where two externae originated, white arrowhead shows scar where single externa originated); F, whole externa, lateral view; G, stalk of externa, showing two cuticular annuli (arrowheads); H, apical view of externa showing presumptive pore (arrowhead) and thalli belonging to an unidentified mesomycetozoean (vertical arrow). Scale bars: A = 400 μm, B = 50 μm, C, E = 1 mm, D, F = 500 μm, G = 100 μm, H = 300 μm.
Remarks.—This is the first record of an akentrogonid from a porcelain crab host. The new species presents only a single annulus on the stalk and no mantle pore (although cyprids are not present and the pore may not have developed yet). One annulus and no mantle pore are characteristics of *Thompsonia*, but the molecular data place the new species closest to *Thylacoplethus magellani*, which has two annuli and a mantle pore. Two other species currently placed in *Thylacoplethus* also have only one annulus: *Thylacoplethus pilodae* and *Thylacoplethus orientalis*. *Thylacoplethus pilodae* on *Pilidius nigrocrinitus* Stimpson, 1859 (Xanthidae) collected from Singapore was described from specimens that contained cyprid larvae and had a mantle pore (Lützen and Jaspersen, 1990). *Thylacoplethus orientalis* was described from *Dardanus impressus* (De Haan, 1849) and *D. arrosor* (Diogenidae) collected from Japan, and was noted to have a large apical pore (Hoeg and Lützen, 1993).

In addition to the clear host differences, *Thylacoplethus porcellanus* differs from *Thylacoplethus orientalis* in lacking a mantle pore (which may correlate with larval development) and in having a conspicuous annulus (as compared to the inconspicuous annulus in *Thylacoplethus orientalis*). The new species is similar to *Thylacoplethus pilodae* in both size and position of the annulus, but it is more tapered proximally (greatest width about 3/4 distance from base of externa vs. halfway from base in *Thylacoplethus pilodae*), and lacks a mantle pore. Based on the molecular data, our specimens are distinct from *Thylacoplethus magellani* (Table 2), but this is the only species of *Thylacoplethus* from which molecular data is known. *Thylacoplethus magellani* Hoeg and Lützen (1993) was cited as *Thompsonia magellani* (sic) by Pérez-Losada et al. (2009), but the species had originally been described within *Thylacoplethus*.

The externae of *Thylacoplethus porcellanus* were covered with thin extensions on the surface that match the morphology of members of the mesomycetozoean group Ecrininae (Fig. 4D), similar to those found on other hermit crab parasites (Williams and Madad, 2010). “Hair-like microuticular projections” have been reported from other akentrogoid externae, e.g., *Thylacoplethus isaeavae* (see Rybakov and Shukalyuk, 2004). These should be re-examined with SEM to determine whether they also represent Ecrininae. In any case, these structures are distinct from the “excrescences” of Boschma (1955).

*Thylacoplethus umanguvatus* n. sp. (Figs. 1C, D, 3B, 4E-H)

Material Examined.—All specimens from *Calcinus morgani*. 15 externae on male host (SL = 3.0 mm) (USNM 1268852, holotype), 56 externae on male host (SL = 2.5 mm), 46 externae on male host (SL = 3.5 mm), 32 externae on female host (SL = 3.5 mm), ~105 externae on female host (SL = 3.2 mm), 35 externae on male host (SL = 3.3 mm), 13 externae on female host (SL = 4.8 mm), 28 externae on male host (SL = 4.8 mm), 85 externae on male host (SL = 3.4 mm), 30 externae on female host (SL = 4.2 mm), 91 externae on male host (SL = 3.2 mm) (USNM1268853-1268862, paratypes in alcohol; USNM1268863, 1268864, paratypes on SEM stub), shallow intertidal, coral rubble, 8°42’S, 115°15’S, 56.82°E, Sanur, Bali, Indonesia, 6 Aug 1997, J. D. Williams coll.

Description.—Immature externae ovoid (0.33 ± 0.02 mm by 0.30 ± 0.04, n = 10), mature externae elongate, up to 1.7 mm in length, 1.1 mm in diameter (1.46 ± 0.13 by 0.97 ± 0.10, n = 10), set on short stalk (Fig. 4E, F). Stalk with two conspicuous annuli (Figs. 3B, 4F, G), basal annulus thinner, distal annulus broad, rounded (Fig. 3B). Mantle thin and transparent, filled with eggs or developing cyprids (Fig. 3B). Spermatogenic bodies in mantle. Minute mantle aperture present subapically in externae with developing cyprids (Fig. 4H), lacking in those with eggs.

In total, up to 100+ externae on carpus, merus, and ischium of pereiopods 1-5 (Fig. 1C, D); larger numbers of externae on pereiopods 4 and 5, some being attached laterally, whereas on pereiopods 1-3 externae are restricted mostly to dorsal and ventral surfaces, and found there in rows.

Remarks.—Five species of *Thylacoplethus* are now known from hermit crab hosts: *Thylacoplethus isaeavae*, *Thylacoplethus magellani*, *Thylacoplethus orientalis* Hoeg and Lützen, 1993, *Thylacoplethus reinhardi* Lützen, 1992, and the new *Thylacoplethus umanguvatus* (see Table 3). *Thylacoplethus orientalis* has only a single annulus, while the other four species have two annuli on the stalk (although very young externae of *Thylacoplethus reinhardi* have only a single annulus; see Lützen, 1992). The mantle pore is subapical and unadorned in *Thylacoplethus isaeavae* and *Thylacoplethus umanguvatus* n. sp., subapical with raised “lips” surrounding it in *Thylacoplethus magellani*, and apical in *Thylacoplethus reinhardi*. Externae are found on the abdomen only in *Thylacoplethus magellani* and *Thylacoplethus reinhardi*, on the pereiopods and abdomen of *Thylacoplethus isaeavae*, and almost entirely on the pereiopods of *Thylacoplethus umanguvatus*. Spermatogenic bodies are found in the mantle in *Thylacoplethus isaeavae* and *Thylacoplethus umanguvatus* (easily seen through the thin cuticle in the mature externae) but are absent in *Thylacoplethus magellani* and *Thylacoplethus reinhardi* as well as in all other species of this genus. In sum, *Thylacoplethus umanguvatus* is most similar to *Thylacoplethus isaeavae*; the two species can be distinguished by host choice, the predominant location of the externae on the body, and the much larger size of externae of *Thylacoplethus isaeavae* (up to 4.9 mm vs. 1.7 mm for *Thylacoplethus umanguvatus*). For a review of the impacts of rhizocephalans on hermit crab hosts see McDermott et al. (2010).

Williams and Boyko (1999) described a new species of bopyrid isopod, *Pseudostegias macdermotti* Williams and Boyko, 1999, associated with the same collection of hermit crabs from Bali. The host hermit crabs were then identified as *Calcinus gaimardii* (H. Milne Edwards, 1848). However, that same year *C. gaimardii* was shown to be a species complex and *C. morgani* was described (Rahayu and Forest, 1999). *Calcinus gaimardii* and *C. morgani* are very similar morphologically and can only be distinguished based on shield coloration or molecular data (McLaughlin et al., 2007; Malay and Paulay, 2009). Upon re-examination of the hosts, we have identified them as *C. morgani* based on coloration of the shield (lacking the red-brown coloration on the anterior portion characteristic of *C. gaimardii*). Additionally, 16S rDNA was 100% similar to *C. morgani* when preliminary
attempts were made to generate sequences of mitochondrial

genes for *Thylacoplethus umanguvatus*.

As reported for *Thylacoplethus porcellanus* (see above),

many externae bore thin extensions from the cuticle that
appear to be Eccrinales rather than cuticular extensions of

the externae.

Ecology.—Of 34 *C. morgani* sampled in Bali, 11 (32.4%) harbored *Thylacoplethus umanguvatus*. Parasitized hosts harbored up to 105 externae per host (48.7 ± 31.6 exter-

nae/host, n = 11). Numerous scars left from previous ex-

ternae or those dislodged during handling were observed on pereiopods 1-5 of all specimens. Some scars represented the point of origination of one externa, whereas others repre-

sented two externae that erupted from the same point (Fig. 4E). In one specimen, two externae were found to erupt from the anterior portion of the pleon, just below the fifth pereiopods; all other externae were recorded on pereiopods 1-5.

Etymology.—The specific name *umanguvatus* is an adjec-

tive and refers to the grape-like appearance of clustered ex-

ternae on the host: *umang* (Bahasa Indonesia for hermit crab) plus *uva* (Latin for grape or berry).

**Discussion**

Three species (including two new to science) of Thompsoni-

dae are described herein based on morphology and, for two of them, partial sequences of the nuclear 18S rDNA gene. Høeg and Lützen (1993) revised Thompsoniidae, while de-

scribing three new genera and seven new species, and re-

vived the genus *Thylacoplethus*, previously considered a ju-

nior synonym of *Thompsonia*. We provide a review (Table 3) of all currently recognized species in the two genera with their respective hosts and geographic ranges (five species of *Thompsonia* and 13 species of *Thylacoplethus*). The rela-

tively high sequence divergence (14.4-21%) between these two genera suggests that *Thylacoplethus* merits its genus sta-

tus despite the problems that still exist in placing species within the genera based on morphology. Until such time as more taxa of *Thompsonia* and *Thylacoplethus* are added to the phylogeny, and the morphology is known in greater de-

tail for more species, the tree is equivocal as to whether these taxa should be considered two genera or only two clades of species within a single genus.

A close relationship between *Thompsonia* and *Thyla-

coplethus* was also supported by Pérez-Losada et al. (2009),

who reconstructed the phylogeny of Theostraconida, the taxon that contains parasitic and suspension-feeding Cirripedia among others, using DNA sequences of three nuclear genes including 18S rDNA, as well as larval characters. These au-

thors were the first to include a species of *Thylacoplethus* in a cirripede phylogeny, although they mistakenly cited *Thylacoplethus magellani* [as *Thompsonia magellana* (sic)] and submitted the GenBank sequence as such. In addition, Peréz-Losada et al. (2009) claimed that their akentrogonid species did not form a monophyletic clade because they in-

correctly considered *Sylon hippolytes* as a kentrogonid (as shown in their supplementary material file); this is incor-

rect morphologically as there is no kentrogon stage in *Sy-

lon* (Høeg and Rybakov, 1992), and it also contradicts Glen-

ter and Hebsgaard’s (2006) molecular study, which had al-

ready shown *Sylon* to group with the Akentrogonida. In our study, we included all akentrogonid sequences avail-

able in GenBank, except that of *Chthamalophilus delagei* Bocquet-Védrine, 1957. This sequence is evidently not reli-

able, as it showed too many differences in the conservative parts of a preliminary alignment that included all akentro-

gonida taxa as well as the kentrogonid *Polyascus polygenea*. Our phylogeny recovered two well-supported clades, one containing *Thompsonia*, *Thylacoplethus*, *Jensia*, *Diplothy-

lacus* and *Boschmaella* (92% posterior probability), and the other containing *Clistosaccus* and *Sylon* (100% posterior probability). *Polyascus japonicus* does not nest in either of these clades but instead forms a polytomy with them. Glenner et al. (2010) supported a monophyletic Akentro-

gonida using 18S rDNA and 28S rDNA sequences with results very similar to ours. However, they did include *C. delagei*, which grouped with *Boschmaella japonica*, for-

ming a monophyletic Chthamalophilidae as a sister taxon to Thompsoniidae. They did not include *Thylacoplethus magellani*, probably because it was not yet available in GenBank from the study by Pérez-Losada et al. (2009).

The new *Thylacoplethus* is the first record of an akentro-

gonid rhizocephalan parasitizing a porcellanid crab. Of the 13 species of *Thylacoplethus*, 8 parasitize anomurans. *Thylacoplethus cubensis* Reinhard and Stewart, 1956 parasitizes a species of the Munididae (Galatheoidae), the family most closely related to Porcellanidae (Bracken-Grissom et al., 2013) also known to be infested with *Thylacoplethus*. The externae of *Thylacoplethus cubensis* and *Thylacoplethus porcellanus* are quite different; those of *Thylacoplethus cubensis* bear two annuli, whereas those of *Thylacoplethus porcellanus* only bear a single annulus.

Our findings of a new host group record for *Thyla-

coplethus* support previous work showing that Thompsoni-
daes is the rhizocephalan family with the widest range of hosts infesting most major groups of decapods, and also two species of Stomatopoda (Høeg and Lützen, 1993; Lützen and Du, 1999; Walker, 2001; Boyko and Williams, 2009; Glen-

ner et al., 2010). The hosts, so far reported for the clade con-

taining *Thylacoplethus*, *Thompsonia* and *Jensia* (= Thom-

psoniidae) (see Tables 2 and 3), include caridean shrimps, brachyuran and anomuran crabs, and stomatopods. Consid-

ering this diverse group of hosts it seems reasonable to as-

sume that the common ancestor of these thompsoniid genera was a generalist parasite, and that speciation was probably triggered by accidental infestation of new host lineages.

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REFERENCES


Yoshida, R., M. Osawa, M. Hirose, and E. Hirose. 2011. A new genus and species infusorium (Chatton and Lwoff, 1927). correct name for this ciliophoran taxon is therefore Pottsia ryderi. As this name is a homonym of both Pottsia Chatton and Lwoff (1927) described the monotypic ciliophoran genus and species Pottsia infusorium. Because of the assumed homonymy between Pottsia Chatton and Lwoff and Pottsia Ryder, Corliss (1960) proposed the replacement name Pottsiocles for Pottsia Chatton and Lwoff. Corliss (1960) could have, and probably should have, considered Pottsia Ryder as a name introduced in synonymy and therefore an unavailable name. However, he did not and Corliss’ (1960) action in erecting a replacement name for Pottsia Chatton and Lwoff made Pottsia Ryder, 1885 available. The correct name for this ciliophoran taxon is therefore Pottsiocles infusorium (Chatton and Lwoff, 1927). Pottsiocles (sic) Edwards and Vevers, 1975 is a spelling error.

References


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