PROJECT DESCRIPTION

1. Introduction and Objectives

The overarching goal of the proposed research is to bring the study of the biodiversity and systematics of aplacophoran molluscs into the 21st century while training the next generation of invertebrate systematists and establishing the PI for a lifetime of excellence in research and education. Aplacophora is a diverse clade of shell-less, worm-shaped marine molluscs (reviewed by Todt et al. 2008, Todt 2013). Several species are ecologically important (Scheltema 1997) and Aplacophora plus Polyplacophora (chitons) make up the sister taxon of all other molluscs (reviewed by Kocot 2013, Giribet 2014). The number of scientists studying aplacophorans has declined in recent years, leaving just a handful of expert researchers worldwide. Meanwhile, almost every time an expert samples a new place, new species are discovered (e.g., Scheltema 1999, Kocot and Todt 2014, Passos et al. 2016) and both known but undescribed species and unidentified specimens collected in environmental surveys (e.g., Schiaparelli et al. 2014) continue to grow in number. Identifying most aplacophorans has traditionally required the destructive process of histology. However, use of micro-computed tomography (micro-CT) scanning, which enables non-destructive visualization of internal anatomy from several specimens simultaneously, would significantly accelerate the pace of specimen identification. Identification of specimens using molecular approaches would also reduce challenges to studying aplacophoran biodiversity and make the group more accessible to non-experts, but this is hindered by a paucity of available DNA barcodes. Because the group holds an important place in molluscan phylogeny and is morphologically variable in terms of many key molluscan characters (reviewed by García-Álvarez and Salvini-Plawen 2007), understanding the evolutionary polarity of these characters in Aplacophora could also shed light on the early evolution of Aculifera and Mollusca as a whole. Unfortunately, phylogenetic analyses to date have never had representative taxon sampling for Aplacophora (reviewed by Todt 2013).

The proposed research aims to address fundamental questions about aplacophoran biodiversity and systematics such as: How many species of aplacophorans are there? How widely are species distributed? How rare or common are most species? Does the current taxonomy of the group reflect its evolutionary history? What can improved understanding of the evolutionary polarity of aplacophoran character states tell us about the last common ancestors of Aplacophora, Aculifera, and Mollusca as a whole? To this end, the PI and his team will employ a novel workflow that collects data using light microscopy, micro-CT, scanning electron microscopy (SEM), and DNA barcoding – all from the same specimen. Further, genome and transcriptome sequencing, target-capture phylogenomics, ancestral character state reconstruction, and molecular clock analyses will be performed to provide a robust and broadly sampled phylogenetic framework, a revised classification system for Aplacophora, and new insight into the early evolution of Mollusca. The specific objectives of this proposal are to:

1. Address the erosion of taxonomic expertise for many small benthic marine invertebrate taxa by hosting workshops to integrate training of the next generation of invertebrate systematists with essential specimen collection.
2. Use an integrative taxonomic approach to identify and describe species, prepare monographs on groups that are particularly understudied or in need of revision, characterize the faunas of select understudied regions, and aid future researchers in identifying specimens by producing a library of DNA barcodes from expert-identified specimens.
3. Reconstruct aplacophoran phylogeny using a genome-enabled target-capture approach with broad taxon sampling and revise aplacophoran taxonomy to reflect its phylogeny.
4. Conduct ancestral character state reconstruction and molecular clock analyses to infer the plesiomorphic states of key morphological characters and timing of major events in molluscan evolution, thus shedding light on the early evolution of the most diverse animal phylum in the sea.

The proposed activities will enable the PI to build a firm foundation for a lifetime of quality research and teaching. The PI is committed to excellence in education as demonstrated by his strong teaching evaluations, mentoring, and outreach activities (see 4. Educational Plan, Biosketch, and Departmental Letter from Janis O'Donnell). This research will directly integrate educational objectives including hosting field-based taxonomy training workshops led by world expert taxonomists and mentoring of a postdoc, graduate students, and a standing team of 5-7 undergraduate researchers. The PI is working with an assessment expert, Dr. Ginger Bishop in the Office of Institutional Effectiveness at UA, to learn and

implement best practices for assessment of the effectiveness of each education and research objective. Knowledge gained from assessments will be used to dynamically improve approaches, maximizing impact and increasing retention of researchers in the field (see Education Plan).

The PI is uniquely qualified and well-positioned for this work as he has been working with aplacophorans for nearly ten years (Kocot et al. 2009, 2011, in prep., Scheltema et al. 2012, Kocot and Todt 2014, Todt and Kocot 2014, Mikkelsen et al. 2018, in review), is an expert in phylogenomics (e.g., Kocot et al. 2011, 2013, 2017, 2018, Moroz et al. 2014, Hall et al. 2017), has significant field experience, collaborates with world experts on diverse taxa, and has amassed a collection of around 5,000 aplacophoran specimens from all over the globe (see below). All facilities, equipment, and resources needed to conduct this research are available to the PI (see Facilities, Equipment, and Other Resources statement).

2. Background and Significance

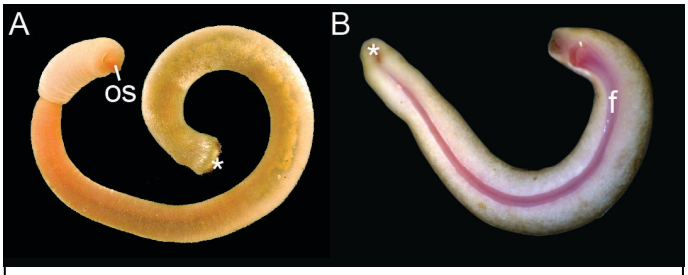

Figure 1. Aplacophoran molluscs. **A.** *Chaetoderma nitidulum* (Caudofoveata). **B.** *Alexandromenia crassa* (Solenogastres). os = oral shield, f = foot, * = mantle cavity. Specimens are ~8 cm in length (A) and 4 cm in length (B).

Taxonomy and systematics are fundamental disciplines in biology that provide information on the diversity of life on Earth, which is essential to the fields of conservation, ecology, and evolutionary biology (Thomson et al. 2018). These fields offer the basis for all comparative studies, providing the names by which we call organisms, and a framework that explains their evolutionary relationships. Confronted with a vast number of undescribed species and high rates of extinction, these fields struggle with a lack of trained personnel and time to discover and characterize biodiversity, sometimes before it is lost forever (Costello et al 2013). New tools such as micro-CT scanning and high-throughput DNA sequencing could revolutionize taxonomy and systematics by dramatically accelerating the pace at which detailed data collection can be performed. However, scientists skilled in cutting-edge approaches that also have traditional taxonomic expertise are needed to advance these fields (Schmeller et al. 2017).

One group with particularly limited taxonomic expertise is Aplacophora, a clade of worm-shaped molluscs that completely lack a shell. Instead, aplacophorans are covered in a dense coat of spiny and/or scale-like calcareous sclerites. These marine animals have a narrow or completely reduced foot, a unique posterior sensory organ, and a small mantle cavity at the posterior-most part of the body. There are two clades of aplacophorans: Caudofoveata (also called Chaetodermomorpha; **Figure 1A**), which burrow and feed on foraminiferans, and Solenogastres (also called Neomeniomorpha; **Figure 1B**), which are epibenthic and generally eat cnidarians (Scheltema 1978, 1993, Salvini-Plawen 2003, Todt et al. 2008, Todt 2013). Both groups are morphologically variable, globally distributed, and ancient (Vinther et al. 2012). Although they are not common at intertidal depths (and are thus unfamiliar even to many zoologists), some are abundant and ecologically important bioturbators in deep-sea habitats (Scheltema 1997) with at least one species reaching densities of up to 350 per m^2 (Scheltema and Ivanov 2009) and densities of >50 per m^2 are not uncommon (e.g., Scheltema 1995, 1997). Although their phylogenetic position has been debated (reviewed by Kocot 2013, Giribet 2014), phylogenomic studies have shown that Aplacophora and Polyplacophora (chitons) form a clade called Aculifera (Scheltema 1993), sister to other molluscs (Kocot et al. 2011, Smith et al. 2011; **Figure 2**). Despite this, knowledge on aplacophoran biodiversity and systematics is exceedingly limited, no aculiferan genomes are available, and the group is rarely considered, even in purportedly comparative studies of molluscan biology (reviewed by Haszprunar 2012).

In recent years, the number of taxonomists working on this already understudied group has dropped significantly. Three of the world experts – Amelie Scheltema, Luitfried von Salvni-Plawen, and Christoffer Schander, who collectively described >1/3 of the ~420 named species – have passed away, and another has left academia. There are now only around five labs worldwide actively working on the group. Two of these PIs are close to retirement and another is leaving academia this year. About 420 species have been described, but the actual number is estimated to be tenfold higher (Todt 2013). For example, only about

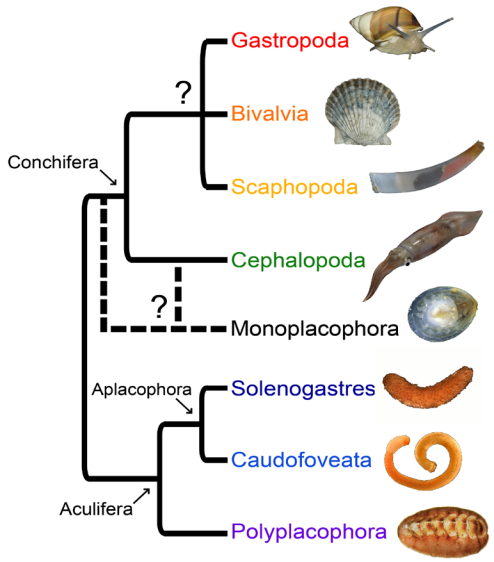

Figure 2. Current understanding of deep molluscan phylogeny based on phylogenomic studies. Aplacophora and Polyplacophora comprise a clade called Aculifera, which is sister to all other molluscs.

ten Australian species have been described but Scheltema (2001) noted 65 morphospecies from just three deep-water surveys that were limited in geographic scope. There are many other "known unknowns" all over the globe. For example, the PI described three species from Friday Harbor, WA (Kocot and Todt 2014), but researchers there have been aware of the existence of their existence since the 1970s (M. P. Morse personal communication). In addition to such obvious diversity, there are likely many cryptic species. For example, an undergraduate researcher in the Kocot lab recently conducted DNA barcoding on "*Wirenia argentea*" specimens from the Northeastern Atlantic and recovered seven species in a genus that was previously known to consist of only two (Hawkins and Kocot unpublished data). Further, distributions of very few species are well-known; most aplacophorans are known only from their type locality, but some well-known species appear to be broadly distributed (e.g., Corrêa et al. 2014, Todt and Kocot 2014). Other significant discoveries are also likely to be made studying this group. For example, the PI recently discovered an Antarctic solenogaster living in glass sponges that completely lacks a foot, radula, and mantle cavity – all the traditional hallmarks of the phylum Mollusca. There is much work to be done, and a new generation of taxonomists needs to be trained to do it.

Aplacophoran classification is based primarily on characters of the aragonitic sclerites, cuticle, radula, foregut glands, and reproductive organs. Sclerites are an important taxonomic characters and, in some cases, a simple glance at sclerites can identify a specimen from anywhere in the world to the genus level (e.g., Scheltema et al. 2012, Kocot and Todt 2014). Unfortunately, sclerites of museum specimens stored in ethanol diluted with slightly acidic water or containing residual formaldehyde that decomposed to formic acid are often completely dissolved, rendering these specimens useless for taxonomic work in most cases. One reason for the paucity of aplacophoran taxonomists may be related to challenges in obtaining the detailed internal anatomical data needed for species-level identification. This is usually done via semi-thin serial histological sectioning of decalcified specimens embedded in plastic resin, which is a relatively laborious process that requires destructive sampling in a manner incompatible with DNA-based work. However, micro-CT scanning could significantly accelerate the pace of aplacophoran taxonomy as it can simultaneously provide detailed anatomical data from multiple specimens scanned in one instrument run (Kocot unpublished data).

In addition to a need to improve understanding of aplacophoran biodiversity, little is known about their evolutionary history and the current taxonomy of the group needs to be evaluated in light of a solid phylogenetic framework. As Aculifera is the sister taxon of all other Mollusca, Aplacophora and Polyplacophora are critical to understanding the early evolution of this diverse and important phylum. Some work has addressed polyplacophoran phylogeny (e.g., Okusu et al. 2003, Sigwart et al. 2013), but a phylogenetic framework for Aplacophora, which would shed light on the evolution of key characters for Aplacophora, Aculifera, and Mollusca as a whole, is lacking. Developmental studies (e.g., Scheltema 1993, Scherholz et al. 2013, 2015, Redl 2014) and paleontological studies (Sutton and Sigwart 2012, Sutton et al. 2012, Vinther 2015, Vinther et al. 2017) indicate that aplacophorans likely evolved from a polyplacophoran-like ancestor, but ancestral conditions of many characters critical to understanding early molluscan evolution remain ambiguous (Salvini-Plawen 2003; Kocot et al. in prep.). Recent work (Mikkelsen and Todt 2018, Mikkelsen et al. 2018, in review) indicates that, within Caudofoveata, most genera and one of the three families are not monophyletic and suggests that characters used to define some of these genera are symplesiomorphies rather than synapomorphies. Solenogastres is divided into two superorders, four orders, and 25 families. However, a genus-level analysis of morphological

characters by Salvini-Plawen (2003) indicated that all but one solenogaster taxon above the family level and several families are not monophyletic. Molecular data are available from few aplacophorans and, because solenogasters have GC-rich nuclear ribosomal genes (Okusu and Giribet 2003), most published solenogaster rRNA sequences are actually chimeras (Meyer et al. 2010). In order to investigate higher-level aplacophoran phylogeny, the PI sequenced and analyzed transcriptomes representing all traditionally recognized orders of Aplacophora (**Figure 3**; Kocot et al. in prep). Although this analysis samples just a fraction of the diversity of the group, it recovers several traditional groupings as non-monophyletic, confirming that aplacophoran taxonomy is in need of major revision. Notably, neither of the solenogaster superorders (Aplotegmentaria and Pachytegmentaria) were recovered and the species-rich order "Cavibelonia," a grouping of solenogasters with hollow sclerites, is polyphyletic.

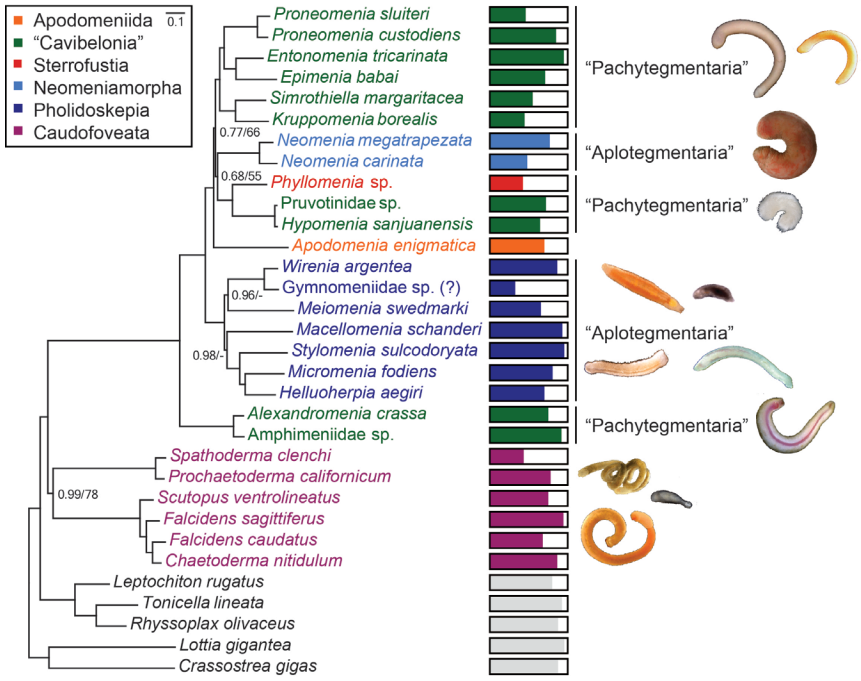

Figure 3. Phylogeny of Aplacophora based on 200 nuclear protein-coding genes. Bayesian inference (BI) topology with posterior probabilities / maximum likelihood (ML) bootstrap support values <1.00/100 presented at each node. Filled bars to right of taxon names represent the proportion of the 200 genes sampled for each taxon. Scale bar = 0.1 substitutions per site.

3. Research Plan

The proposed research seeks to revitalize the study of aplacophoran molluscs while addressing the dire need for taxonomy training on this and other groups of small, benthic marine invertebrates. To this end, the PI and his team will 1) hold three meiofauna and small macrofauna diversity and taxonomy training workshops and an Antarctic research cruise to collect essential specimens for this research while providing experiential learning opportunities for budding taxonomists and systematists, 2) describe >50 new species, prepare monographs for select taxa, characterize the faunas of select regions, and aid future researchers in identifying specimens by producing a library of DNA barcodes, 3) reconstruct aplacophoran phylogeny and molluscan class-level phylogeny using target-capture phylogenomic approaches, and 4) infer ancestral states of key characters for Aplacophora, Aculifera, and Mollusca as a whole and conduct molecular clock analyses to infer the timing of key cladogenic events in molluscan evolution. All of the proposed research activities will directly incorporate training of young scientists and foster career development for the PI, establishing a firm foundation for a lifetime of high-quality invertebrate biodiversity and systematics research and education (see Educational Plan and Career Implications and Long-Term Vision).

The research team will be led by Dr. Kocot (PI) and consist of one postdoctoral researcher (M. Carmen Cobo Llovo), two Ph.D. students, one M.S. student, and a standing team of 5-7 undergraduates. The PI will organize workshops, train and mentor the postdoc and students, conduct taxonomic work, write manuscripts, present at meetings, conduct outreach, and oversee all aspects of the project. The postdoc is already experienced in working with aplacophorans (e.g., Cobo et al. 2012, Pedrouzo et al. 2014, Cobo 2017; see Biosketch). She will help train students in morphological techniques, conduct monographic work on Pholidoskepia, and receive training in genomic and bioinformatic approaches. The Ph.D. students will be encouraged to explore their own questions within the scope of this proposal, but both will be expected to become expert invertebrate zoologists with dissertation chapters focused on aplacophoran biodiversity and/or taxonomy in addition to phylogenetics and genomics. Most likely, one Ph.D. student will lead genomics and protein-coding gene-based phylogenomics and the other Ph.D. student will lead ultraconserved element (UCE)-based phylogenomics, ancestral state reconstruction, and molecular clock analysis. The M.S. student, who has already started working in the PI's lab, will identify and describe aplacophorans from New Zealand. Undergraduates will use light microscopy, SEM, micro-CT, histology, and barcoding to identify and describe specimens and produce a reference DNA barcode database. Students will also participate in outreach such as lab activities at local high schools and events at UA.

To date, the PI has assembled a nearly complete library of aplacophoran taxonomic literature and a diverse collection of nearly 5,000 aplacophoran specimens representing ~150 species from across the globe. The PI has large collections from Iceland (~3,500 specimens from the IceAGE cruises; Brix et al. 2014), and New Zealand (~500 specimens from NIWA and Museum of New Zealand Te Papa Tongarewa). Smaller numbers of specimens from waters off Antarctica, Italy, Japan, Norway, the U.S. and elsewhere are also in-hand (another ~1,000). Most of these are suitable for both morphological and molecular work and the PI has written permission to destructively sample specimens as needed. While this collection spans much of the recognized diversity of Aplacophora, it lacks or has limited sampling for some phylogenetically important and/or particularly diverse taxa of interest (e.g., Chaetodermatidae, Dondersiidae, Hemimeniidae, Lepidomeniidae, *Limifossor*, Sandalomeniidae, and Sterrofustia).

Objective 1: Address the erosion of taxonomic expertise for many small benthic marine invertebrate taxa by hosting workshops to integrate training of the next generation of invertebrate systematists with essential specimen collection.

This objective aims to collect essential specimens for this research while building taxonomic expertise on understudied invertebrates and fostering international collaborations. Proposed are three meiofauna and small macrofauna diversity and taxonomy training workshops and an Antarctic research cruise. The workshops will bring together ten to fifteen students and around ten world experts on understudied invertebrates who will serve as mentors. Workshops will follow highly successful models such as the 2010 Smithsonian Meiofauna Biodiversity and Taxonomy Workshop, at the Smithsonian Tropical Research Institute in Bocas del Toro, Panama. The PI has already organized a network of taxonomists with diverse organismal expertise from six countries who are enthusiastic to serve as mentors: Holly Bik, Johanna Cannon, M. Carmen Cobo Llovo, Peter Funch, Gonzalo Giribet, Ken Halanych, Christoph Held, Rick Hochberg, Ulf Jondelius, Katharina Jörger, Francesca Leasi, Susan Middleton, Trish Morse, Jon Norenburg, Gustav Paulay, Greg Rouse, Megan Schwartz, Ashleigh Smythe, Martin Sørensen, Billie Swalla, and Rob Toonen (see Letters of Collaboration). Sampling at previous collection localities of important taxa will be conducted in tandem with training in taxonomy, techniques, and career development. Students will learn from mentors via both lectures and one-on-one lab work while collaboratively working to sort, document, and preserve specimens to the benefit of all participants' research programs. Details on student selection, teaching materials, assessment, and how retention of students in the field will be maximized are presented in the Education Plan.

In the summer of year 1, the PI will organize a 16-day meiofauna and small macrofauna biodiversity and taxonomy training workshop at the Smithsonian Marine Station (SMS) in Fort Pierce, FL (see Letter of Collaboration from SMS Director Valerie Paul). The PI and several of the mentors have sampled the area previously and already know several rich and diverse sampling sites, making it an excellent place for the first workshop. This workshop will take a taxon-survey approach where a team of ten experts will teach ten students practical skills in the collection, identification, characterization, and preservation of meiofauna and small macrofauna. The second workshop will be a five-week summer course at Friday

Harbor Labs (FHL) taught in year 2 by the PI and Jon Norenburg (Smithsonian Institution National Museum of Natural History) called "Techniques of Taxonomy: Biodiversity and Systematics of Marine Invertebrates in the 21st Century" (see Letters of Collaboration from FHL Director Billie Swalla and Jon Norenburg). This course will build on the model of the SMS workshop and emphasize the synthesis of traditional taxonomy and advances in technology. Up to fifteen students in the FHL course will train in the taxonomy of diverse invertebrates from the two instructors and eight other invited mentors. Further, students will undertake independent projects with opportunities to learn and employ SEM, histology, confocal microscopy, micro-CT scanning, DNA barcoding, transcriptome sequencing from single meiofaunal animals, and relevant bioinformatic analyses (e.g., assembly, annotation, orthology inference, phylogenomics, etc.). The third workshop will take place in late November of year 3 at the Hawaii Institute of Marine Biology (HIMB) in Kaneohe, Hawaii (see Letter of Collaboration from Rob Toonen). This workshop will follow the model of the SMS workshop with ten students and ten mentors. Students will also learn about ongoing work at HIMB employing autonomous reef monitoring structures (ARMS) and mesocosms. Notably, field work conducted by Jon Norenburg and Katharina Jörger in 2016 identified several excellent meiofaunal sampling sites and an incredible diversity of solenogasters with at least 7 species (all new to science) being collected in just a few days.

In addition to the proposed workshops, a 21-day cruise sampling off Argentina and the Antarctic Peninsula with *R/V Lawrence M. Gould* is requested late in year 2. Sampling sites were selected on the basis of type localities and other collection records for essential taxa (e.g., Salvini-Plawen 1978, García-Álvarez and Urgorri 2003, García-Álvarez et al. 2009, Salvini-Plawen and Paar-Gausch 2004), mostly in the vicinity of the South Shetland Islands. The Antarctic Peninsula is a hotspot for aplacophoran biodiversity with forty-seven species described and many more "known unknowns." There is virtually no material from this region available in museums suitable for molecular work and much of the available material is decalcified and thus not even suitable for morphological work. Antarctic field work is important to the success of this project as several unsampled but phylogenetically important taxa are well-known from this region. Sampling will primarily be conducted at depths of 200 to 500 meters with epibenthic sleds, trawls, dredges, box corers, and grabs, depending on substrate type. Sample sieving, sorting, and processing will follow a "cooling chain" (Riehl et al. 2014) to facilitate observation and documentation of living specimens and ensure that samples collected are suitable for molecular work. Samples that cannot be sorted live in a timely manner at-sea will be bulk-fixed in buffered 95% ethanol for future sorting in the lab. The PI is aware that ship time is very expensive and has a large carbon footprint. The duration of the proposed cruise was carefully calculated to ensure success while minimizing cost. In order to increase the impact of this cruise on the general public as well as other scientists not on board, photographer Susan Middleton will join as a mentor to take and disseminate photos of animals via social media and subsequent gallery exhibitions (see Letter of Collaboration). Susan's beautiful portrait-style photos of animals (e.g., Middleton 2014) will be a valuable resource for outreach and building awareness of Antarctic marine invertebrate biodiversity. Additional details on the requested ship time are presented in the Logistical Requirements and Field Plan.

For all workshops and the cruise, representatives of virtually all species collected will be imaged live and specimens will be preserved with buffered 95% ethanol, buffered formalin, RNAlater, freezing at -80°C, or otherwise as appropriate. Sampling will be opportunistic and specimens of as many diverse invertebrates as possible will be collected and preserved for ongoing and potential future research projects using the most appropriate preservation strategy for each taxon. Specimens will be accessioned into the Alabama Museum of Natural History Invertebrate Zoology collection with all data including images and other metadata managed with ARCTOS (see Data Management Plan).

The proposed workshops and cruise will yield essential specimens for this research. Additionally, these will be formative experiences for early-career systematists and will undoubtedly lead to collaborations among mentors and students. To facilitate such collaborations and share the PI's novel integrative taxonomic workflow (see below) with researchers studying other groups, the PI will maintain contact with workshop and cruise participants via social media and an e-mail listserv and invite participants to his lab to train in using this workflow on their taxa of interest. Additional details on the proposed workshops can be found in the Educational Plan, Logistical Requirements and Field Plan, and UNOLS Ship Time Request.

Objective 2: Use an integrative taxonomic approach to identify and describe species, prepare monographs on groups that are particularly understudied or in need of revision, characterize the faunas of select understudied regions, and aid future researchers in identifying specimens by producing a library of DNA barcodes from expert-identified specimens.

The pace of Aplacophoran biodiversity research has been constrained by the effort required to identify specimens. To remedy this problem, the PI has developed a new workflow (**Figure 4**) where histology, which is a relatively slow, laborious, and destructive process that is incompatible with molecular work, is replaced with micro-CT scanning (when possible, see *Histology* below) to rapidly visualize internal anatomy in a non-destructive manner (Faulwetter et al. 2013) compatible with downstream techniques such as SEM and DNA extraction.

Figure 4. Proposed data collection workflow. Specimens are imaged using stereo light microscopy with extended focal imaging. Specimens are then contrasted and imaged using micro-CT scanning. Subsequently, specimens are dried and imaged using low acceleration voltage SEM. DNA is then extracted (sclerites are retrieved and mounted on a slide as a permanent voucher) for barcoding or sequencing library preparation.

Micro-CT: The PI's team will employ micro-CT to visualize internal anatomy. Specimens will be stained with 1% iodine or 2.5% molybdic acid in ethanol and scanned with a Bruker SkyScan 1173 (spatial resolution of 7 µm), which is suitable to collect data on internal anatomy from all but the tiniest specimens (<2 mm in length). However, UA is currently negotiating acquisition of a FEI HeliScan (spatial resolution of 800 nm), which will be used once available. One advantage of micro-CT is that multiple specimens can be scanned simultaneously, increasing throughput relative to histology while reducing cost. Specimens will be stabilized in tubes with pieces of 0.5% agarose or cheesecloth and labelled with 3D-printed plastic numbers so that they can be easily distinguished from each other in post-processing. Parameters for imaging will be optimized based on specimen size using the smallest pixel size possible. NRecon, CTAnalyzer, and CTVox (Bruker) will be used to process images and render 3D models. As proof of concept, the PI used micro-CT to examine the internal anatomy of some average-sized (~1 cm) solenogasters (**Figure 5A**). All characters needed to identify these specimens can be clearly seen.

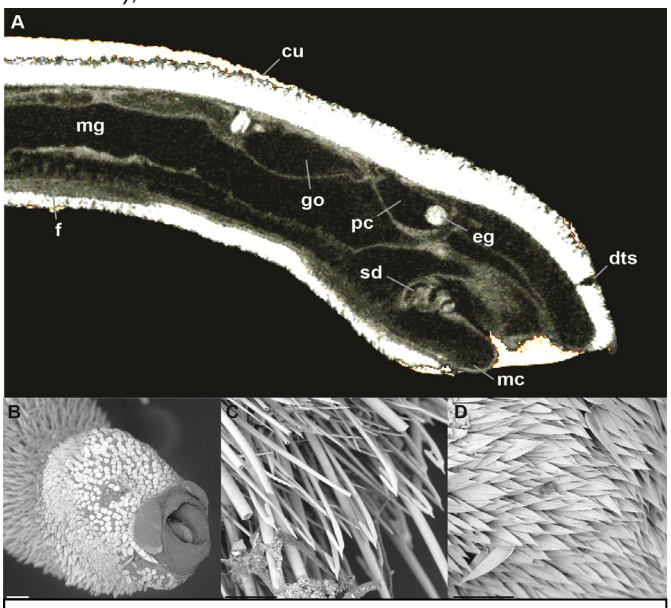

Figure 5. A. Oblique micro-CT section through posterior of *Entonomenia tricarinata*. cu = cuticle with sclerites, dts = dorsoterminal sensory organ, go = gonad, mg = midgut, sd = spawning duct, pc = pericardium, mc = mantle cavity, eg = egg. **B-D.** Scanning electron micrographs showing sclerites of *Spathoderma alleni* (**B**), *Pruvotina longispinosa* (**C**), and *Wirenia argentea* (**D**).

SEM: Specimens will be air-dried and imaged (uncoated) using the Kocot lab Phenom Pro table-top SEM under low vacuum with a low accelerating voltage (5 kV; (**Figure 5B-D**). Subsequently, samples will be put directly into lysis buffer for DNA extraction. The Kocot lab routinely uses this approach and consistently recovers high-quality DNA. Thus, specimens preserved in 95% ethanol, which strikes a good balance between preserving DNA and

morphology, can be used in this workflow incorporating stereo light microscopy, micro-CT, and SEM followed by high molecular weight DNA extraction and preparation of permanent microscope slides of isolated sclerites as a voucher – all from the same specimen.

Histology: One potential challenge stems from limitations to the resolution and contrast differentiation of micro-CT. If micro-CT scans do not provide adequate detail (as is likely for specimens smaller than 2 mm in length) or if a new species is being described, histological sectioning will be used to study internal anatomy. Histology will be carried out by the postdoctoral researcher, Ph.D. students, and advanced undergraduate students following routine approaches (e.g., Kocot and Todt 2014, Todt and Kocot 2014). For the description of new species, three-dimensional anatomical reconstructions will be assembled with AMIRA (FEI) following the approaches of Klink et al. (2015).

DNA Barcoding: DNA barcoding will be conducted by undergraduate researchers led by the postdoc and Ph.D. students with oversight of the PI. DNA will be extracted using the Omega Bio-tek E.Z.N.A. MicroElute kit. Intact sclerites will be isolated after lysis and preserved in buffered ethanol for later slide-mounting, thus providing a permanent morphological voucher for each specimen. Aplacophoran-specific COI and 16S primers and PCR protocols that work well have already been established (Goble, Hawkins, and Kocot unpublished data). Data analysis will follow Mikkelsen and Todt (2018). The Barcode of Life Database (BOLD) Student Data Portal (Ratnasingham and Hebert 2007) will be used for downstream analyses such as species delimitation approaches and barcode gap discovery.

Leveraging this workflow, the PI and his team will complete several tasks addressing specific problems and questions encompassed by this objective. Minimally, the following will be undertaken:

A. Monography of Pholidoskepia: Pholidoskepia is an order of 60 described species of small-bodied, scale-bearing solenogasters (García-Álvarez and Salvini-Plawen 2007) that is strongly supported as monophyletic by phylogenomics (Figure 3). Examination of in-hand material has revealed that this group is much more diverse than currently recognized. The postdoctoral researcher, who worked with this group for her Ph.D., will advance understanding of the diversity of Pholidoskepia by preparing a global-scale monograph with description of many new species and a synopsis of the diversity of group. This work will generate hypotheses about evolutionary relationships, character trait evolution, and biogeography and will inform taxon sampling for subsequent phylogenetic studies.

B. Monography and Revisionary Systematics of Amphimeniidae: The PI's unpublished phylogenomic results show that Amphimeniidae is sister to all other (sampled) Solenogastres (Figure 3). Thus, this group is of particular interest. The family currently consists of 10 named genera and 28 species, but the monophyly of these genera is dubious. One of the Ph.D. students will analyze in-hand, newly collected, and museum material to revise this over-split family and collect data on key morphological characters from the sister taxon of all other Solenogastres. If DNA barcodes are unable to resolve relationships within this family, this work will be conducted in parallel with Objective 3 (see below).

C. Biodiversity Inventories: Most aplacophoran species are known only from their type locality. However, few large-scale biodiversity inventories have considered aplacophorans (but see García-Álvarez et al. 2014) and several species are known to be widely distributed (e.g., Corrêa et al. 2014, Todt and Kocot 2014). In order to expand on the paucity of knowledge on Aplacophoran biodiversity, the graduate students will characterize the aplacophoran faunas of select geographic regions that are particularly diverse (the Antarctic Peninsula; Salvini-Plawen 1978), understudied (Hawaii and New Zealand), or both (Iceland; Goble et al. unpublished data). In addition to simple species presence/absence information based on epibenthic sled and trawl samples, quantitative samples collected during the proposed cruise by box corer will be used to shed light on species abundance, relative abundance, richness, faunal similarity among stations, and bathymetric distribution following Kaiser et al. (2009) and Brandt et al. (2009). This work will be done in collaboration with other cruise participants to maximize impact on understanding of benthic invertebrate biodiversity of each region.

D. Ecological Niche Modelling: Armed with detailed data about the distributions of aplacophoran species in these regions, we will conduct ecological niche modelling to predict the current and future distributions of aplacophoran species. Environmental data and species occurrence records will be curated in an ArcGIS project. Modeling methods will follow the approaches of Meißner et al. (2014).

E. Development of a Reference DNA Barcode Library: DNA barcoding would be the fastest and most reliable method of aplacophoran identification if a representative reference database were available.

However, just nineteen aplacophoran cytochrome *c* oxidase subunit I (COI) and seven large subunit mitochondrial rRNA (16S) sequences are available on NCBI. Therefore, undergraduate researchers led by the postdoc will produce a library of COI and 16S barcode sequences for around 384 expert-identified specimens per year including multiple individuals of each putative species.

F. Honoring Final Contributions: Amelie Scheltema was a world expert on aplacophorans who was an active researcher up until her death. The PI inherited much of her library and laboratory notes including a stack of folders labelled "MSs in draft worth finishing." The PI and his team will obtain the relevant museum material and work to complete some of the most significant manuscripts addressing questions about aplacophoran taxonomy, biodiversity, and evolution.

This work will provide answers to basic questions about the biodiversity of these understudied animals and result in 7-10 publications ranging in scope from large-scale biodiversity analyses and revisionary systematic works to single species descriptions and regional studies led by undergraduates. Although description of ~50 species is small relative to the estimated >4,000 species, this is an honest assessment of what can be accomplished during the award period and will increase the number of named aplacophorans by >10%. The taxonomic and biodiversity inventory work proposed here will lay the foundation for substantial future contributions in this area, which will remain a core activity of the PI's lab.

Objective 3: Reconstruct aplacophoran phylogeny using a genome-enabled target-capture approach with broad taxon sampling and revise aplacophoran taxonomy to reflect its phylogeny.

This objective aims to build on the PI's preliminary data to produce a broadly sampled reference phylogeny for Aplacophora and re-examine deep molluscan phylogeny using a cost-effective target-capture phylogenomic approach. To this end, existing transcriptome data and two newly sequenced aplacophoran genomes will be employed by the Ph.D. students to design probes to sample and analyze hundreds of protein-coding genes and ultraconserved elements (UCEs) from around 200 aplacophorans plus UCEs from another ~50 molluscs broadly spanning the higher-level diversity of the group. These numbers are based on current understanding of the diversity of Aplacophora and Mollusca and the anticipated number of new species to be described during the course of the project.

Marker Selection and Probe Design: To select protein-coding genes suitable for inferring aplacophoran phylogeny, the PI curated nucleotide alignments for 8,251 orthologous genes following Kocot et al. (2013). This list will be pared down by one of the Ph.D. students to exclude genes with high branch-length heterogeneity, high compositional heterogeneity, or low phylogenetic signal using TreSpEx (Struck 2014), BaCoCa (Kück and Struck 2014), and MARE (Meyer and Misof 2010), respectively. Subsequently, 120 bp probes tiled at 60 bp intervals will be designed using BaitFisher (Mayer et al. 2016). For investigation of aplacophoran and molluscan class-level phylogeny using UCEs (Faircloth et al. 2012), probes will be designed using the PHYLUCE pipeline (Faircloth 2015). A preliminary analysis using this approach with ten publicly available mollusc genomes plus outgroups yielded 4,759 UCEs sampled for at least 2/3 of the taxa. Maximum likelihood analysis of the best-sampled 1,000 loci resulted in a strongly-supported tree consistent with the current understanding of molluscan phylogeny (Pabst and Kocot unpublished data). Notably, only UCEs will be used to investigate molluscan class-level phylogeny because three large-scale studies using protein-coding genes have been brought to bear on this question but were unable to confidently place some key taxa (Kocot et al. 2011, Smith et al. 2011, Kocot et al. in prep.).

Genome and Transcriptome Sequencing & Annotation: Probes must be filtered to include only those that target conserved exons and UCEs with clear orthology across Mollusca. Because no genomic data are available from any aplacophoran, high-quality genomes will be sequenced for the solenogaster *Neomenia megatrapezata* (haploid size = ~700 Mbp) and the caudofoveate *Scutopus ventrolineatus*, which are both already in-hand. The genome size of *S. ventrolineatus* could not be measured with in-hand material, but all aplacophorans examined to date have a haploid genome size between 0.3 to 700 Mbp (Kocot et al. 2015, Kocot unpublished data). High molecular weight DNA will be extracted from frozen hemocytes from one individual (*Neomenia*) or one entire starved animal (*Scutopus*) using the Genomic Tip kit (Qiagen) and treated with RNAse. DNA will be sent to The University of Arizona Genomics Core Facility for PacBio library preparation and Sequel sequencing to 30X coverage and to Macrogen (Cambridge, MA) for Illumina TruSeq PCR-free library preparation and 2 X 150 bp paired-end (PE) sequencing using two lanes of an Illumina HiSeq X (>100X coverage). Multiple assembly approaches will be explored (Bankenvich et

al. 2012, Zimin et al. 2013, Walker et al. 2014, Koren et al. 2017). Optical mapping will be performed using the Saphyr platform (BioNano) to improve scaffold contiguity. To facilitate genome annotation, transcriptome data will be generated and assembled as per Kocot et al. (2018) and genome annotation will be performed following the approach of Hall, Kocot, and Baughman et al. (2017). Sequencing two genomes will increase the cost of this task, but having aplacophoran genomes will be essential for probe design and, even with the added cost of genome sequencing, this approach is still much cheaper than transcriptome-based phylogenomics, which is not feasible due to a lack of suitably preserved material for most taxa. These reference genomes will be important for identifying conserved exons, excluding probes for protein-coding genes that span splice sites, confirming orthology of the captured sequences, and establishing filters against contamination.

Target-Capture Phylogenomics: DNA extracted for barcoding (Objective 2) will be sheared to ~350 bp using a Covaris M220. Dual-indexed sequencing libraries will be prepared with the NEBNext Ultra II DNA Library Prep Kit. Probe synthesis and 6-12-plex hybridization will be outsourced to Arbor Biosciences (Ann Arbor, MI). Sequencing of captured libraries to a depth of ~8 million reads per sample (~50 per lane) on a HiSeq 4000 lane with 2 X 150 bp PE sequencing will be outsourced to Macrogen. A modified version of the approaches of Teasdale et al. (2016) and Hamilton et al. (2016) will be followed to select a subset of the BaitFisher probes and PHYLUCE will be used to design probes for UCEs. Around 500 loci of each type will be targeted. Subsequent dataset assembly will follow established approaches (e.g., Teasdale et al. (2016) for protein-coding genes and Faircloth (2015) for UCEs). Partitioned matrices will be analyzed following approaches used routinely by Kocot (e.g., Kocot et al. 2017). Maximum likelihood (ML) analyses will be conducted with RAxML (Stamatakis 2014) using the best-fitting model for each gene with ≥100 bootstrap replicates. Because bootstrapping in a phylogenomic context has been criticized for sometimes providing support for artifacts (Salichos and Rokas 2013, Lemoine et al. 2018), supertree approaches such as internode certainty (Salichos et al. 2014) and ASTRAL (Mirarab et al. 2014) will also be explored. Bayesian inference (BI) analyses will be performed using Phylobayes MPI 1.5a (Lartillot et al. 2013) using the CAT-GTR+Γ4 model with 4+ chains run until a bpcomp value <0.3 indicates convergence.

This work is poised to provide a well-resolved phylogenetic framework for Aplacophora and Mollusca, enabling much-needed taxonomic revision and improving understanding of the evolution of this diverse phylum. Results will facilitate future work in the PI's lab using phylogenetic comparative methods (Huelsenbeck and Rannala 1997) to test evolutionary hypotheses. For example, although most solenogasters are feed on cnidarians, certain clades eat annelids, nemerteans, bivalves, bryozoans, and even sea spiders (Okusu & Giribet 2003, Kocot unpublished data, Jörger personal communication). Future work in this area will investigate co-evolution of solenogaster taxa and their prey (Hafner and Nadler 1988) and the potential of host-switching as a driver of diversification rate (Krug et al. 2015).

Objective 4: Conduct ancestral character state reconstruction and molecular clock analyses to infer the plesiomorphic states of key morphological characters and timing of major events in molluscan evolution, thus shedding light on the early evolution of the most diverse animal phylum in the sea.

Perhaps more than any other animal group, understanding of early molluscan evolution has been constrained by the notion of a generalized body plan rather than the consideration of individual characters (see Lindberg and Ghiselin 2003 and references therein). Understanding the plesiomorphic character states of Aplacophora, Aculifera, and Mollusca as a whole could help improve understanding the origin and early evolution of the second most diverse animal phylum and several enigmatic fossil taxa (reviewed by Vinther 2015). Aplacophora is a morphologically plastic group in terms of many characters thought important to understanding molluscan evolution (e.g., characters related to the radula, body shape, pericardium, musculature, and reproductive and digestive systems), but inference of the plesiomorphic states of these characters has been hindered by the lack of a robust phylogenetic framework for the group. Therefore, in light of published and newly generated morphological data and the broadly sampled phylogenetic framework produced for Aplacophora in Objective 3, ancestral character state reconstruction will be conducted for key molluscan characters.

Some aplacophoran characteristics (e.g., worm-shaped body, posterior sense organ, and reduced mantle cavity) are most parsimoniously interpreted as synapomorphies. However, other aplacophoran features may represent retained plesiomorphies that have been lost or modified in other molluscs. For example, as

adults, most molluscs generally have a broad, rasping radula with multiple teeth per row whereas several putative stem-group fossil molluscs and most aplacophorans have a narrow radula with two teeth per row (distichous; Scheltema et al. 2003). This condition is also observed during development in some chitons and gastropods (Scheltema 1993). However, species in five genera of Solenogastres have a broad radula with multiple teeth per row (García-Álvarez and Salvini-Plawen 2007) and the phylogenetic position of these taxa is unknown. If the plesiomorphic condition of the aplacophoran radula is actually broad, and the distichous radula is paedomorphic (Schletema 1993), this has important implications for interpretation of the fossil record and evolution of the radula. Likewise, the organization of the reproductive system varies dramatically among aplacophoran taxa and molluscan classes, but whether this variation can shed light on the ancestral state for Mollusca (or even Aplacophora) depends on understanding it in the context of the group's evolutionary history. Other key characters for which the ancestral conditions are unclear and the aplacophoran condition may be informative relate to the pericardium (vestige of the coelom), musculature, and digestive systems (Salvini-Plawen 1985, Kocot et al. 2011). Therefore, although aplacophorans are not "basal" as previously thought (Salvini-Plawen 2003), understanding evolution of aplacophoran characters could provide substantial insight into early molluscan evolution.

Molecular clock analyses by Vinther et al. (2012) indicate that modern aculiferans appeared in the Ordovician. However, the analyses by Vinther et al. (2012) were based on just seven housekeeping genes with data from just two aplacophorans. Further, some of the topologies recovered by that study are at odds with the emerging consensus on deep molluscan phylogeny (Kocot et al. 2011, 2017, in prep. Smith et al. 2011). Specifically, some analyses recovered Aplacophora paraphyletic and others recovered Cephalopoda sister to Aculifera (rather than in a clade with rest of Conchifera), prompting Vinther et al. (2012) to state "Thus, details of the origin and evolution of Aplacophora remain to be assessed." Therefore, in light of the phylogenetic reconstructions generated in Objective 3, the timing of key events in early molluscan evolution will be re-examined using a molecular clock.

Methods: The utility of ancestral character state reconstruction analyses for understanding the evolutionary history of individual traits is well-documented (e.g., Händeler et al. 2009, Cartwright and Nawrocki 2010, Kocot et al. 2011). A morphological character matrix will be assembled by one of the Ph.D. students building on published data matrices (Haszprunar 2000, Salvini-Plawen 2003, Kocot et al. 2011) with new data collected during the course of this research. Morphological character data will be mapped onto the reconstructed phylogenies produced in objective 3 and ancestral character state reconstruction will be performed using Bayesian stochastic mapping of character evolution (Nielsen 2002, Huelsenbeck et al. 2003) in R (R Core Team 2000) using the phytools package (Revell 2012). Molecular clock analyses will be performed based on the reconstructed phylogenies produced in Objective 3. Multiple approaches employing BEAST 2 (Bouckaert et al. 2014) and Phylobayes 3 (Lartillot et al. 2009) will be compared following the approaches of Barba-Montoya et al. (2017) and Vinthet et al. (2012), respectively. Fossil calibrations will build on the set used by Vinther et al. (2012) testing the effect of using putative stem-group aculiferans (e.g., Sutton and Sigwart 2012, Sutton et al. 2012, Vinther et al. 2017) as calibration points. The joint time prior used will be carefully inspected using sensitivity analyses as advocated by Barba-Montoya et al. (2017).

By employing new data from aplacophorans and these established methods, this work is poised to provide a new understanding of the evolution of key molluscan characters and the timing of major events in molluscan evolution. Thus, this objective will extend the impact of this work beyond aplacophoran biodiversity and systematics and inform all studies on molluscan and early animal evolution.

4. Educational Plan

Institutional Context: UA is a public student-centered research university and the flagship university of Alabama. UA emphasizes experiential learning and has a long history of serving the people of Alabama, leveraging the experience of its faculty and the energy of its students. In line with the mission of UA and the PI's career goals, this educational plan will directly integrate training of students and a postdoc with the proposed research activities.

Workshop Participants: The PI will organize three meiofauna and small macrofauna biodiversity and taxonomy training workshops and a research cruise with student participants. Workshops will follow the

successful models of others organized previously by Jon Norenburg and courses the PI has previously taken and taught at FHL. During workshops, students from Alabama and across the globe will develop taxonomic expertise on understudied benthic marine invertebrates, learn laboratory and field techniques, and collect specimens for their research. As a graduate student, the PI attended a similar workshop in Panama and took the Marine Invertebrate Zoology course at FHL and found both to be formative educational experiences that also provided him with the opportunity to collect valuable research specimens. Workshops such as these form lasting partnerships among senior scientists and graduate students, stimulate additional interactions through mutual connections, and result in large-scale proposals and important publications. Workshops will be advertised broadly via EvolDir and other lists, targeted e-mails to colleagues, and social media. Student applicants will submit their CV, a letter of recommendation, and a statement explaining their background, career goals, and reasons for wanting to participate. Successful applicants will be selected by a 3+ mentor panel on the basis of adequate background training, relevant career goals, and an enthusiasm for the study of invertebrates. Training materials (presentation slides, identification guides, keys, protocols, etc.) will be compiled by the PI and other mentors, largely building on existing resources developed for previous successful workshops. These documents as well as recordings of taxonomist lectures and other related resources will be freely disseminated via the PI's website to provide benefit to others beyond the select few able to participate in the workshops. **Assessment:** Assessment of the effectiveness of all educational objectives of this proposal will be conducted with assistance from Dr. Ginger Bishop in the UA Office of Institutional Effectiveness (see Letter of Collaboration). Mentors will work closely with students throughout the workshops and assess student knowledge and skills near the beginning and end of each workshop using a rubric. Daily "plus/delta" surveys will be used to assess what aspects of the workshop participants find is going well and what could be improved. At the end of the workshops, students will present results of their studies and collections as well as collaboratively assembled species lists. Mentors will assess the efficacy of the training objectives by critique of presentations based on a rubric and all participants will provide constructive feedback. Immediately after each workshop, the PI will follow up with students and mentors with a post-assessment survey to gauge how participants felt the workshop could be improved in the future. The PI will also invite workshop and cruise participants to visit his lab to learn his integrative taxonomic workflow and apply it to their group of interest. Over the longer term (>5 years), the PI and mentors will maintain contact with students to foster collaborations, continue to mentor them in the field, and assess the impact of these workshops on the long-term retention of scientists in the field.

Undergraduates: Funding for this proposal will support the mentoring of a standing team of at least five undergraduate researchers at a time in a manner that has proven to be highly effective for teaching laboratory, presentation, and writing skills while generating valuable data. Undergraduate researchers will learn organismal biology and skills in light microscopy, SEM, histology, and/or molecular techniques and will directly contribute to specimen documentation and DNA barcoding. Advanced students will take on independent projects that reflect their interests and skills. Undergraduate research in the Kocot lab is organized as a course (BSC 398) with enrolled students conducting research for credit (not pay). As such, students are expected to be contributors to published work. Thus, undergraduate researcher commitment in the lab is expected to be a minimum of six hours per week with continuity of research over a minimum of four semesters. Undergraduate researchers will also participate in weekly lab meetings and have opportunities to attend scientific meetings. This activity is particularly enthusiastically supported by and advances the educational and research goals of UA, the College of Arts and Sciences, and the Department of Biological Sciences (see Departmental Letter from Janis O'Donnell). UA places significant value on experiential learning and has instituted numerous programs designed to promote undergraduate research including the UA Learning in Action Quality Enhancement Plan, the Office of Experiential Learning, and the Office of Undergraduate Research. The PI will also continue to enrich his Invertebrate Zoology and Principles of Biology II courses by incorporating discussion of his research into his regular lectures. **Assessment:** The PI will develop a rubric to assess the impact of his undergraduate researcher mentoring program on student knowledge and skills each semester, making improvements as needed. Impact of undergraduate research will also be assessed through student participation in conferences (e.g. the Undergraduate Research and Creative Activity Conference at UA), authorship on manuscripts, and, ultimately, completion of their degree and entry into the STEM workforce or enrollment in graduate/professional school. The PI will keep in touch with former undergraduate researchers long-term (>5 years) to assess the impact of this experience. The PI assesses effectiveness of his teaching in

undergraduate courses using quizzes, active learning activities (e.g., think-pair-share), posing open questions to the class, writing assignments, and pre-assessment questions that are re-tested in exams.

Graduate Students: With guidance and assistance from the PI and postdoc, graduate students will train in organismal biology and both traditional as well as cutting-edge techniques to discover, identify, describe, and catalog invertebrate biodiversity. Graduate students will also learn molecular and bioinformatic techniques. The Ph.D. students will learn to assemble and annotate eukaryotic genomes, infer the phylogeny of Aplacophora using phylogenomics, and conduct ancestral character state reconstruction. Graduate students will also be expected to mentor undergraduates, present research results at meetings, and lead and co-author publications to disseminate results. **Assessment:** Graduate student progress will be assessed through successful completion of milestones in the program (such as coursework and qualifying exams), presentations at conferences, authorship on peer-reviewed publications, and, ultimately a smooth transition to a postdoctoral or other position in-line with the student's interests and career goals.

Postdoc: The PI has already been in contact with M. Carmen Cobo Llovo (see Letter of Collaboration, Biosketch), a young expert on aplacophorans who is enthusiastic about joining his lab. Since the beginning of 2018, Carmen has been awarded a Fulbright Ruth Lee Kennedy Travel Bursary, a grant from the Malacological Society of London, and a grant from the Society of Systematic Biologists to support a collaborative research visit to the Kocot lab in 2019. Carmen's dissertation focused on the biodiversity of a particularly challenging group of solenogasters. In the Kocot lab, Carmen will be a colleague in training who will leverage her taxonomic expertise to advance knowledge on aplacophoran biodiversity and evolution. Moreover, Carmen will significantly expand her molecular and bioinformatic skillset, gain valuable experience by helping to manage the lab and mentor students, present at national and international meetings, publish papers, and develop new collaborations. See the Postdoctoral Mentoring Plan for details on training and assessment of the postdoc.

The PI: Finally, this work will provide valuable learning opportunities for the PI himself. The PI anticipates learning a great deal through interactions with world expert taxonomists during the proposed workshops, which will likely develop into fruitful future collaborations. Senior taxonomic experts, station managers, and ship's crew will undoubtedly be valuable teachers to the PI and other relatively early-career mentors, providing advice and consultation on successfully leading a training workshop and/or cruise. CAREER funding will support hiring a postdoc with expertise in aplacophoran taxonomy. The PI will leverage this opportunity to work closely with this young expert to exchange knowledge and further develop his own taxonomic expertise. Finally, funding for this work will allow the PI's lab to establish and implement a number of laboratory and data analysis techniques (e.g., target-capture phylogenomics and optical mapping) in his lab for the first time, adding these tools to his lab's growing technical repertoire.

5. Career Implications and Long-Term Vision

The proposed objectives of this 5-year project (**Figure 6**) will support the early development of the PI's academic career, helping him to establish a life-long pursuit of knowledge on the biodiversity and systematics of aplacophorans and other important but understudied marine invertebrates. Further, the integrated educational objectives proposed will enable the PI to share his enthusiasm with others by working closely with a postdoc, students, and workshop and cruise participants. Although many of these interactions will be relatively temporary (workshops end, postdocs move on, students graduate, etc.), the PI will make these the first of many such training activities focused on developing human resources in taxonomy and systematics. The education of a large team of undergraduate researchers and teaching courses and workshops in invertebrate zoology at UA and at marine stations will continue as a permanent part of the PI's education activities. Aside from the explicit objectives of this proposal, which will establish the PI to work on aplacophorans for many years to come, this work will undoubtedly lead to new discoveries and questions in addition to the proposed research, which will further fuel the development of the PI's career. In particular, interactions with world expert taxonomists and students during workshops and the cruise are especially likely to lead to additional collaborations and the chances of this team making significant discoveries both within and beyond the scope of this proposal are high.

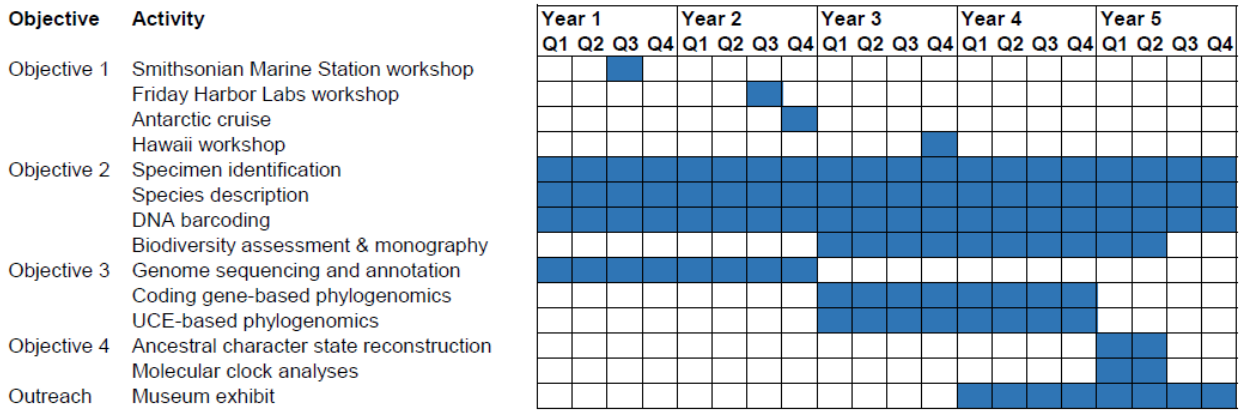

Figure 6. Proposed timeline for the duration of this five year award.

Intellectual Merit

The proposed research and educational plan will resurrect research on Aplacophora in the U.S. and revolutionize the way small invertebrate biodiversity research is conducted through implementation of a novel specimen identification and characterization workflow employing rapid, high-tech approaches as well as traditional tools. Using this workflow, the PI and his team will discover and characterize aplacophorans in particularly diverse and understudied geographic areas, describe new species, and write monographs for select clades in need of revision. This work will also produce a DNA barcode library from hundreds of expert-identified specimens to help future researchers accurately identify specimens and detect cryptic species. Taken together, these outcomes will benefit the field by improving understanding of the diversity and distribution of poorly known but ecologically important members of marine subtidal habitats. In addition to discovering and characterizing aplacophoran biodiversity, the evolutionary history of the group will be inferred using hundreds of molecular markers. In order to conduct target-capture phylogenomic work, the first aplacophoran genomes will be sequenced and annotated. This is an important prerequisite for probe design, but will also provide valuable genomic resources to the scientific community. These resources will be of interest to researchers in diverse fields ranging from systematics to comparative genomics and will stimulate other significant research activities. Further, deep molluscan phylogeny will be examined using UCEs for the first time. Finally, in light of a well-resolved phylogeny for Aplacophora, the inaccurate taxonomy of the group will be revised, the evolutionary polarity of key molluscan characters will be inferred with ancestral character state reconstruction, and the timing of key events in molluscan evolution will be inferred using molecular clock analyses, thus shedding light on the early evolution of this ancient and diverse phylum and the identity of several enigmatic fossils.

6. Broader Impacts of the Proposed Research

The proposed research stands to revolutionize the way small invertebrate taxonomy is conducted with an innovative workflow that can easily be applied to diverse taxa. Because Mollusca is the second most species-rich animal phylum and the subject of thousands of research papers in diverse fields every year, this work will be of great interest to malacologists and invertebrate zoologists. Improved understanding of aplacophoran biodiversity, resolving aplacophoran phylogeny, and retracing the steps of early molluscan evolution will also inform fields such as ecology, paleontology, and genomics, just to name a few. In addition to the proposed activities, this work will stimulate other research activities and collaborations beyond the scope of this proposal. Most notably, with large teams of taxonomic experts sampling remote and/or understudied areas, the possibility of significant discoveries is high. Results of this work will be disseminated widely through presentations at domestic and international meetings by the PI, postdoc, and students including organized symposia at international meetings. Specifically, the PI will organize a symposium on aplacophorans at the World Congress of Malacology in 2022. This symposium will involve student research from the PI's laboratory as well as student and mentor participants from the workshops. This work will also lead to a substantial number of publications (>15) in high-quality, peer-reviewed journals. All morphological and molecular data generated will be made easily accessible in public databases (see Data Management Plan) and specimens will be deposited in appropriate museums.

As described in detail above, this work will integrate taxonomy training for early-career taxonomists/systematists and training in multiple areas for members of the PI's lab. Further, facilitated by Alabama Science in Motion (see Letter of Collaboration from Lisa Clark), the PI and/or members of his lab will visit at least three Tuscaloosa area high schools per year, most of which are primarily attended by underrepresented minorities, and educate 9th grade biology students about systematics and the tools used in this field (especially SEM). This grade-level was chosen because this material will integrate well with the existing 9th grade biology program and enrich it. The goal of this program is to expose students to the science of systematics and its hypothesis-driven pursuit to perceive, describe, and explain diversity in an organized and useful manner. The PI will bring an SEM 'kit' with enough supplies for all students in the class, teach students how to mount specimens, and conduct an SEM demo by operating his lab's SEM via a remote desktop connection. Students will write a paper about a question that tools used in systematics could address and prepare a biological specimen of their own for SEM. At the end of the program, students will be expected to be familiar with the science of systematics and be able to formulate questions using tools used in the field. Student papers will be evaluated by their teacher, the PI, and at least one other member of his lab using a rubric. This rubric will also be used to identify the top four papers. These students and their teacher will be invited to visit the PI's lab in the Alabama Museum of Natural History to image their and their classmates' specimens with a table-top SEM, and practice basic molecular techniques. Once established, this program will be continued indefinitely to enhance science education in the region. The PI will also continue to regularly engage with area K-12 students and the public through guest lectures and host at least one high school student in his lab per year as part of the Scientist for a Day program. The PI will also continue to engage in other forms of educational outreach such as blogging during workshops and the cruise, posting images of organisms collected during field work to the web and social media, and taking advantage of any additional outreach opportunities to share his enthusiasm on topics related to this work.

The general public will be educated through an exhibit at the Alabama Museum of Natural History. This exhibit will include infographics explaining and illustrating the methods and techniques used in this research and the importance of taxonomy. Additionally, interactive video displays with micro-CT scans and high-magnification images of aplacophorans and other invertebrates illustrating various microscopic techniques and diagnostic characteristics of the organisms will be made. A skilled scientific illustrator and graphic designer has been recruited to help develop a visually appealing and effective exhibit (see Letter of Collaboration from Megan Rock). Further, a video explaining and demonstrating micro-CT will be developed in collaboration with museum director Dr. John Friel (see Letter of Collaboration) and presented on a large display adjacent to the exhibit and uploaded to the web. This exhibit will be integrated into the museum's existing physical web beacon system, which will assess reach by logging visitors and functioning as an interactive guide.

During the course of all of the proposed research and educational activities, the PI will make a genuine effort to recruit women and individuals from underrepresented groups. Specifically, the PI will reach out to high-achieving women and students from underrepresented groups in his courses, the Louis Stokes Alliance for Minority Participation program, the UA Emerging Scholars Program, and the UA Randall Research Scholars Program. Also, most of the area schools to be visited by the PI are predominantly attended by African Americans. Through the proposed teaching and mentoring activities, the PI aims to contribute to the education of a new generation of systematists as well as that of other individuals that, while they may go on to other fields, will hopefully retain an appreciation for and understand the value of biodiversity and systematics.

Finally, the PI will continue to be involved in numerous synergistic activities ranging from serving as a reviewer for numerous peer-reviewed journals and granting agencies and an associate editor for *Marine Biodiversity* to serving as the treasurer and president elect of Capstone Alliance, an organization focused on community, outreach, and pursuing advocacy in support of the LGBTQ+ community to make UA a safe, inclusive, and supportive campus (see Biosketch).

7. Results from Prior NSF Support

Kocot is a beginning investigator without prior NSF support.