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The Malacologist

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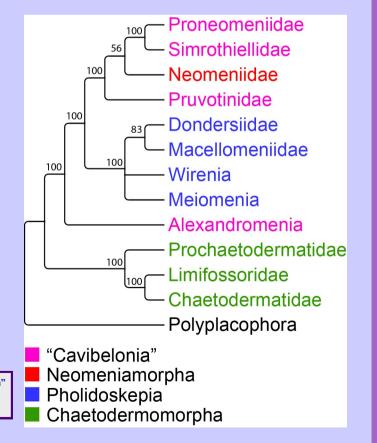
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"This preliminary molecular phylogeny of Aplacophora drastically differs from the morphology-based taxonomy of this interesting but often overlooked group. Molecular phylogenetics practically turns upside-down previous hypotheses of phylogenetic relationships in both Neomeniomorpha (a cavibelonian taxon as the first branch within the clade) and Chaetodermomorpha (Prochaetodermatidae as the first branch within the clade). "

> See "Preliminary molecular phylogeny of Aplacophora" by K. M. Kocot¹, C. Todt², N. Mikkelsen² & K.M. Halanych¹ on page 8



EDITORIAL

Please accept my apologies for the lateness of this issue.

This 61st issue of *The Malacologist* (the Bulletin of the Malacological Society of London) focuses on research grant reports. For many years, the Malacological Society of London has supported researchers, mainly students, by giving financial support for both travel and separately, research. Applications are carefully and fairly scrutinized by a panel of Council members. One of the provisos of an award is that awardees should write a short piece for *The Malacologist*, describing the outcome of the award. Most awardees diligently do this, though a few do not and we don't hear from them again. Sometimes, gratifyingly, acknowledgments appears in journals other than the *Journal of Molluscan Studies* (see below). The eclectic mix of reports in *The Malacologist* and articles in the *Journal of Molluscan Studies* are a sign of an interesting and healthy field of study. Long may it continue. For example, the front page of this issue highlights an eye-catching report by a research grant awardee Kevin Kocot (and colleagues) suggesting a possible fundamental revision of the taxonomy of the Aplacophora.

While this issue was in preparation, the World Council of Malacology (WCM) took place in the Azores, ably organised by Antonio de Frias Martins. Hopefully it will be possible to include a report in the next issue of *The Malacologist*. A list of For those who have registered on the WCM website at www.wcm2013.com (it is easy to do), the programme and a list of posters are available. The WCM meetings are a triennial highlight, allowing us to catch up on research in a range of malacological fields other than our own and importantly, allowing us to meet old and new friends. Here is a comment from a young malacologist whose travel to the WCM was supported by a Malacological Society of London travel grant.

"When my advisor M. Malaquias first told me that I should attend the world congress and present some of my work my first thought was "What am I going to contribute, when all these well-known scientists are attending?". I felt this was going to be like a small fish (slug) suddenly finding itself in a great ocean populated by giants. As the congress started I felt quite out of place, however as the days went on I started to talk with people, and everybody seemed like friends you have known forever. And as always in chaos involving people you usually catch the surname last.

Realising that some of the people you have been introduced to or went to dinner with one night was actually the scientific superstars you have cited, respected and to some degree feared or that the kind and funny people you met the other day, have surnames like Gosliner, Kocot, Krug, Schrödl, Sigwart or Wägele, to name a few, is an experience in itself. Additionally to be able to pick and choose, and sometimes regrettably have to choose between, brilliant presentations of the freshest results in our field served by the most prominent scientists of our age is well worth the trip.

My experience at the WCM2013 in the Azores, new friends, connections, and what new students of malacology should expect of meetings like this can best be summarised by the words of Manuel's friend Jesus (who turned out to be J. S. Troncoso). "I was like you, and you will be like me, everybody here (malacologists) are a family, and you are a part of this family." I can say for my part it truly felt like this was the case. No matter what I felt before the congress, I will be coming back if given the opportunity.

Trond R. Oskars, Master's Student Phylogenetic Systematics and Evolution Research Group University Museum of Bergen, Norway

All such acknowledgments of the help given by the Malacological Society of London are greatly valued by the Council. It makes our efforts worthwhile and increases the desire in the Council to give more help.

TAXONOMIC/NOMENCLATURAL DISCLAIMER

This publication is not deemed to be valid for taxonomic/nomenclatural purposes [see Article 8b in the International Code of Zoological Nomenclature 3rd Edition (1985), edited by W.D. Ride *et al.*].

Prof. Emeritus Georges Dussart Canterbury Christ Church University, Kent, CT1 1QU UK

Funding Acknowledgment

María Bagur, C.A. Richardson, J. Gutiérrez, L.P. Arribas, M. Socorro Doldan & M. Gabriela Palomo (2013) Age, growth and mortality in four populations of the boring bivalve *Lithophaga patagonica* from Argentina. *Journal of Sea Research* **81** (2013) 49–56

"This study was financed by the projects CONICET PIP 0732, ANPCyT PICT 1338, and a <u>Travel Grant to M.B. from the Malacological Society of London.</u> The bivalves in the protected area of Puerto Pirámides were collected with permission from the Chubut Province (*Project Taxonomía y biogeografía de las especies terciarias y actuales de la familia Mytilidae en Argentina*).....etc"



Research grant reports

What are the C and K corpuscles of apple snails?

Taryn E. Takebayashi

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INTRODUCTION

Apple snails, in particular *Pomacea canaliculata* have a negative impact on wetland agriculture by feeding on crop plants such as rice and taro (Cowie 2002) and are capable of modifying wetland ecosystems by reducing biodiversity and increasing water turbidity (Carlsson *et al.* 2004, Qiu and Kwong 2009). As a result, they have attracted considerable research effort recently, and of particular interest is the finding of putative endosymbionts in the gut of a number of species, with most research focused on *P. canaliculata*. The putative endosymbionts of *P. canaliculata* have been termed C and K corpuscles (Castro-Vazquez *et al.* 2002, Vega et al. 2005, 2006, 2007, 2012a, Koch et al. 2006, 2009). These corpuscles were earlier described as green spherioles (C corpuscles) and brown excretory concretions (K corpuscles) (Macmunn 1900, Andrews 1965) based on morphological observations. Because the corpuscles originated in the midgut and were found in the faeces, they were thought to have digestive and excretory roles (Castro-Vazquez et al. 2002). In this study, the presence of C and K corpuscles was confirmed in two additional apple snail species, *Pila conica* and *Asolene spixii*. Their morphological features were characterized using light, confocal, and transmission electron microscopy (TEM) and their chemical composition was analysed via energy-dispersive x-ray spectroscopy.

METHODS

Light and Confocal Microscopy

Unstained fecal samples for confocal microscopy were viewed on an Olympus Fluoview 1000 Laser Scanning Confocal Microscope. To check for autofluorescence of chlorophyll (present in plants and cyanobacteria), the samples were excited at 488nm. Midgut samples were prepared in the same manner as TEM samples (see TEM methodology below) and stained with uranyl acetate. Midgut samples were viewed under a compound microscope and pictures taken with a digital camera.

Transmission Electron Microscopy

Prior to dissection of the midgut, all test snails were relaxed in tap water with a pinch of menthol crystals for 1.5 − 2 hours. Snails were dissected in 2.5% glutaraldehyde/0.1 M Sorenson's Phosphate buffer, pH 7.4 and post-fixed in 1% osmium tetroxide/0.1M Sorenson's buffer. Subsequently, the tissue samples were dehydrated in a series of graded ethanol changes and propylene oxide and infiltrated with a series of graded LX112 epoxy resin:propylene oxide changes. Finally, the tissue was embedded in pure resin. Ultrathin (≤100 nm) sections of the resin-embedded samples were cut with a diamond knife and double stained with uranyl acetate and lead citrate. TEM grids were viewed and photographed on a Hitachi HT7700 transmission electron microscope (100kV) with an AMT XR41 4 megapixel camera.

Chemical analysis

Energy-dispersive X-ray spectroscopy (EDS) was used to determine the presence or absence of various elements in the corpuscles. EDS was performed as a secondary analysis of TEM prepared midgut sections with a Hitachi S-4800 Field Emission Scanning Electron Microscope with Oxford INCA X-Act EDS System.

RESULTS AND DISCUSSION

Light microscopy, confocal microscopy, TEM, and EDS revealed morphological and chemical features in the C and K corpuscles that may be inconsistent with them being endosymbionts. Light micrographs of the feces and midgut show approximate locations of the corpuscles (Fig.1-2).

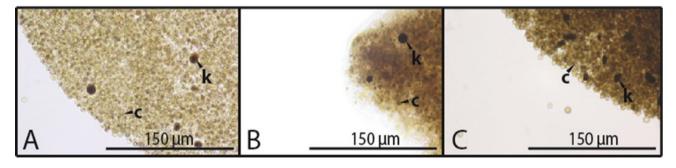


Fig. 1. Light micrographs of snail feces containing corpuscles. A-C – faecal pellets containing corpuscles from *Pomacea canaliculata* (A), *Asolene spixii* (B), and *Pila conica* (C).

Confocal microscopy revealed the absence of autofluorescence from the K corpuscles and a highly varied distribution of chlorophyll in the C corpuscles, even within the same fecal pellet (Fig. 3). TEM also revealed considerable variation in the inner sections of the K corpuscles, including crystalline structures and dark precipitations that were not previously reported (Figs. 4-6). Under TEM, the C corpuscles exhibited a smaller range of forms than the K corpuscles. However, the two most common types (Fig. 7) were quite different from each other. A lack of phycocyanin and thylakoids in either the C or K corpuscles ruled out the possibility of them being cyanobacterial in origin. EDS gave a rough overview of the elements found in the corpuscles, including calcium, iron, silicon, niobium, and gold. Although the iron may have originated from another source, the snails in this study were being fed a diet primarily of spinach, which contains high levels of iron. The presence of the other metals in addition to the high variation in the corpuscles also seems to point towards an external source.

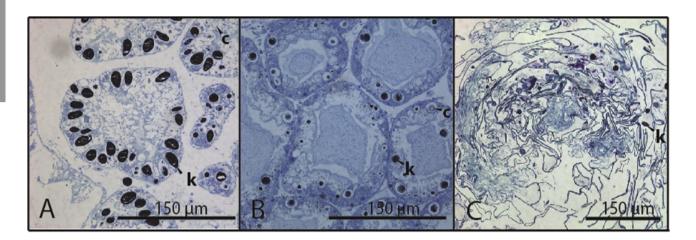


Fig. 2. Light micrographs of snail midgut containing corpuscles. A-C – Light micrographs (40x) of thin sections of the midgut of a *P. canaliculata* adult (A) and an *A. spixii* adult (B), and of *P. conica* feces (C) with K corpuscles (k) and C corpuscles (c).

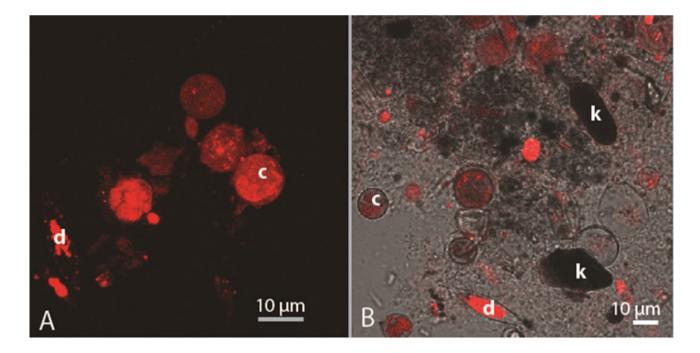


Fig. 3. Confocal micrographs of C and K corpuscles from single fecal pellets of P.canaliculata. A – Note the high variation in chlorophyll distribution in the C corpuscles (c) (as shown by areas of red autofluorescence at 488 nm). B – There is a lack of autofluorescence in the K corpuscles (k) while other material in the pellets, such as diatoms (d), fluoresce brightly.

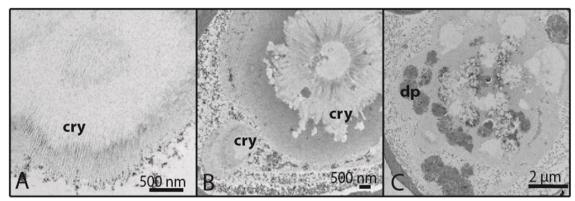


Fig. 4. Variation in the contents of K corpuscles in a single A. spixii. Dark precipitations (dp) and crystalloid structures (cry) appear to be inorganic material. Note lack of thylakoids

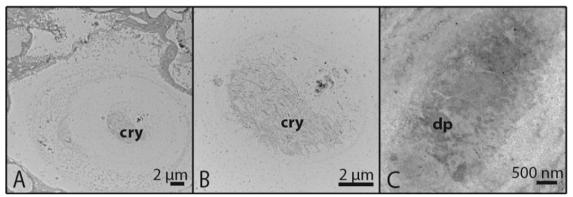


Fig. 5. Variation in the contents of K corpuscles in *P. conica*. A, B – The same K corpuscle with B showing a zoomed in view of the inorganic, crystalloid-like structure (cry). C – Inner contents of another K corpuscle showing features similar to dark precipitations (dp) in the K corpuscles of *A. pixii* (Fig. 4C).

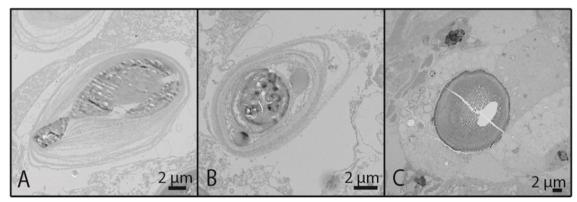


Fig. 6. Variation in the contents of K corpuscles. Some K corpuscles had solid centers of inorganic material. A, B – Two K corpuscles from *P.canaliculata* with hard centers of differing composition. C – End view of K corpuscle from *A.spixii* with a hard center that chipped while the diamond knife cut through it.

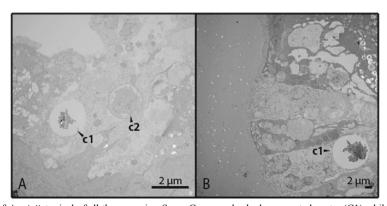


Fig. 7. C corpuscles of *A.spixii*, typical of all three species. Some C corpuscles had a separated center (C1) while others were more uniformly granular (C2). C corpuscles also lacked thylakoids, similar to the K corpuscles.

The true nature of the corpuscles remains unclear. Vega *et al.* (2012a) considered at least the C corpuscles to be endosymbionts, but related to plant chloroplasts, rather than cyanobacteria as previously thought (Vega *et al.* 2006). Based on the present study, a tentative alternative hypothesis is that both the C and K corpuscles may be products of digestion and toxin removal rather than endosymbionts. A review of literature on the hepatopancreas (midgut) of other organisms suggested some mechanisms that might explain the presence of the metals in the K corpuscles. Rebecchi *et al.* (1996) confirmed that the digestive gland of gastropods is important in the storage of calcium and glycogen, and the detoxification process. A study of Struthiolariidae, a family of marine gastropods, referred to greenish spherioles near the basal end of digestive cells as "non-assimilable residue after intracellular digestion" (Morton 1951), and their true nature will only be revealed by further research Both Castro-Vazquez *et al.* (2002) and Morton (1951) may have been studying the same types of spherioles since they both cited Macmunn (1900), who described excretory spherioles containing chlorophyllous pigments. However, Morton asserted that the pigment is from intracellular digestion of food. However, unpublished research by Castro-Vazquez and colleagues continues to investigate the putative endosymbiotic nature of the corpuscle's source.

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In Collembola, the midgut serves as a mineral storage organ in which toxins are trapped in calcium containing granules (Humbert 1978). Brown (1982) described two types of calcium granules in the midgut of Collembola: 1) those containing pure calcium and 2) those containing calcium mixed with other metals including magnesium, manganese, zinc, lead, and iron. This description also appears to be consistent with the midgut gland of the apple snails as there were calcium granules that were shown to be pure calcium by EDS, and the K corpuscles had varied compositions of different metals including iron along with calcium. Simkiss (1981) stated that high concentrations of metals may have a toxic effect on snails and that toxins accumulate as insoluble deposits that are excreted. If the midgut of apple snails is also involved in both digestion and detoxification, it seems plausible that the corpuscles could be part of the digestive and/or detoxification process. This suggestion seems consistent with the observations of Vega *et al.* (2012b) that the corpuscles bioconcentrate heavy metals from contaminated water.

ACKNOWLEDGEMENTS

I thank Tina Carvalho and Marilyn Dunlap of the Biological Electron Microscope Facility (Pacific Biosciences Research Center) at the University of Hawaii at Mānoa. I also thank Robert Cowie, Clifford Morden and Alison Sherwood, for guidance. David Christopher and Carole Hickman provided additional help and support and Kenneth Hayes helped with the initial project proposal. I thank Alfredo Castro-Vazquez for discussion. This research was funded by the University of Hawaii Undergraduate Research Opportunities Program, the Malacological Society of London, and an NSF MRI grant DBI1040548 that was used to acquire the Hitachi HT7700 TEM at the Biological Electron Microscope Facility at the University of Hawaii at Mānoa.

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Population dynamics of the zebra mussel *Dreissena polymorpha* in a redeveloped freshwater dock and the ecological consequences

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INTRODUCTION

Over the past century, the zebra mussel *Dreissena polymorpha* Pallas have spread from their native range in the Ponto-Caspian region to a large area of Europe and North America. Owing to their ability to remove large quantities of suspended matter from the water column and their habit of colonising any available hard surface (including intake pipes and submerged machinery) they are listed as one of the hundred most damaging invasive species worldwide (Lowe, *et al.*, 2000). Their filter feeding abilities have not always been viewed negatively however, and in the early 1990s there was some interest in their use as a biocontrol agent (Reeders & de Vaate, 1990). As such, the introduction of zebra mussels in Salford Quays (hereafter SQ), Greater Manchester in 1992 was seen as a valid solution to water quality problems. The quays were formed by the isolation and artificial mixing of Manchester Docks in the late 1980s but suffered from intense blooms of *Planktothrix agardhii* from 1990 to 2001 (Williams, 2010). Unfortunately, little subsequent work has been done on their filtration efficiency or population dynamics and it remains a possibility that zebra mussels are responsible for autumnal blooms of *Microcystis aeruginosa* since 2004. Densities in 2010 were reported to reach 5500 individuals m⁻² but with an average size of only 15mm, compared with 20-30mm in the rivers Thames and Lee and 22mm for the population originally introduced from Goole (Bellamy, 1997). We were therefore interested in their overall filtration capacity in SQ and whether it is sufficient to control algal production, influence phytoplankton community structure or exert a density dependent effect on the mussels themselves. We also wanted to find out the rate at which colonisation occurs in SQ as an inference for the rate of population spread.

METHODS

Filtration rate experiments followed a modified methodology of Kraak (1994) on mussels of either 15 or 22mm. Cultured algae were used at concentrations of: *Chlamydomonas reinhardtii* 30,000 cells ml⁻¹; *C.reinhardtii* 1,000 cells ml⁻¹; *Planktothrix agardhii* 500 cells ml⁻¹; and a mixed community of: 20% *Cryptomonas ovata*, 60% *Cyclotella sp.* and 20% *C. reinhardtii* 500 cells ml⁻¹. This allowed comparison with previous studies and an assessment of both past and present effects on SQ to be ascertained. Community data were combined with dive surveys conducted in 2010 to obtain a filtration capacity for each basin. Colonisation plates were constructed from 300x150mm slate tiles fixed to wooden boards and suspended at depths of 0, 3 and 6m. These were placed in several areas around SQ and left over a twelve month period.

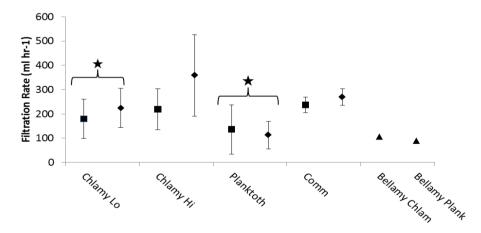
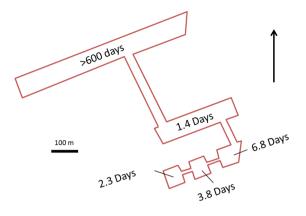


Fig. 1. Filtration rates and standard deviation for mussels of size 15 mm (squares) and 22 mm (diamonds) for different algal groups. Filtration rates for the originally introduced population are denoted by triangles. Starred data indicate significant difference.

RESULTS

There was no significant difference between 15 and 22mm mussels (Fig.1; P>0.1; type II unweighted two-way ANOVA, interaction term not significant, P>0.5), however there was a significant difference between rates in species assemblages (P<0.05). Posthoc analysis shows that significant differences are present between high concentration *C.reinhardtii* (mean= 275 ml hr⁻¹ SD = 128) and *P.agardhii* (mean = 122 ml hr⁻¹ SD = 66). The community assemblage showed no significant change in species composition (chi sq. P>0.05).



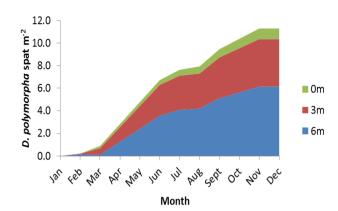


Fig. 2 Turnover time in each area of SQ assuming average size of 15mm

 $Fig.\ 3.\ Average\ spat\ density\ over\ time\ on\ colonisation\ \ ates\ around\ SQ.$

There was clearly a high capacity for filtration by *D. polymorpha* in SQ (Fig. 2) with the exception of the larger northern basin where the surface area to volume ratio is large and few mussels were present.

Fig. 3 shows that spawning principally occurred during the spring and autumn with a notable cessation in the summer and low overall settlement. Colonisation was impeded in the shallower areas by dense growths of macroalgae, principally *Cladophora* spp.

DISCUSSION

The resident *D. polymorpha* population filtered at a capacity that was within the centre of the range given by Walz (1978) and it would also appear that *P. agardhii* inhibits zebra mussel filtration rate, as was seen by Naddafi, *et al.* (2007). This may mean that initial colonisation may have been slow and could partly explain why algal numbers did not decline for some years after introduction. Interestingly there was no increase in filtration rate seen at low phytoplankton concentrations as was seen by Kryger & Riisgård, (1988). No evidence of selectivity was noticed in the community data although analysis of pseudofaeces production would be required to identify if rejection had taken place. With such high turnover rates in the majority of the quays, it would be difficult for algal production to keep pace with consumption. This would only be compounded by additional losses caused by zooplankton and settling rate, in addition to the reduction in production by the low nutrient levels present in SQ. It is suggested that at water temperatures of ≈16°C, limitation of phytoplankton production by *D. polymorpha* is highly likely but the converse may also be true and the reduced average size seen in SQ would seem to be a direct result of overpopulation by *D. polymorpha*.

Density dependence would also explain the low levels of settling on colonisation slates, as adult mussels actively compete with larvae for food and will also engage in infanticide during early life stages (Strayer & Malcom, 2006). Such an effect may lead to a 3-5 year population cycle where a maturing cohort dominates until senescence opens up new resources for maturing larvae (Strayer & Malcom, 2006). If such high degree of limitation does occur then an increase in the incidence of *M. aeruginosa* blooms is possible, especially as nutrient levels continue to fall (Sarnelle, *et al.*, 2012)

CONCLUSION

D. polymorpha may have been slow to colonise SQ but now appear to be capable of large-scale effects on the local environment and intraspecific competition. Unfortunately their effects have become so pronounced that it is impossible to estimate the rates of colonisation seen in SQ, however, we are now planning a molecular investigation to determine if their spread was rapid or incremental. This will also serve to determine if their absence in the northern basin is due to a lag in on-going colonisation or environmental limitation. Further, we also intend to investigate how their filtration rate varies over a yearly cycle by repeating these experiments at varying temperatures. This can then be compared with annual chlorophyll cycles to determine the effect on seasonality. Finally we are also working with past data to try to ascertain when exponential growth occurred. These data will be combined with my present PhD work to determine the overall seasonal succession in SQ and ultimately form part of a predictive model to be used in future management work. A paper entitled "The spread and seasonal effects of the invasive bivalve D. polymorpha in Salford Quays" will be published in the near future.

ACKNOWLEDGEMENTS

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A preliminary molecular phylogeny of the Aplacophora

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INTRODUCTION

Aplacophorans are marine molluscs characterised by a narrow or completely reduced foot, a unique dorsoterminal sensory organ, and a small mantle cavity restricted to the posterior-most part of the body. As the name suggests, aplacophorans completely lack a shell. Instead, they are covered in a dense coat of spiny and/or scale-like calcareous sclerites. There are two distinct lineages of aplacophorans: Caudofoveata (also called Chaetodermomorpha) and Solenogastres (also called Neomeniomorpha). The two groups can most easily be distinguished by the complete lack of a foot in caudofoveates whereas solenogasters have a narrow foot. The biology of these groups was recently reviewed by Todt (2013).

The two clades of aplacophorans have generally been regarded as early-branching molluscs and therefore have been central to questions surrounding the early evolution of the phylum (reviewed by Kocot 2013). Whether these two groups constitute a monophyletic taxon, Aplacophora (e.g., Scheltema 1993), or a paraphyletic grade (e.g., Haszprunar 2000, Salvini-Plawen,2003) has been intensely debated (reviewed by Todt *et al.* 2008, Todt 2013). Recent molecular studies (Kocot *et al.* 2011, Smith *et al.* 2011, Vinther *et al.* 2011) have shown that the two lineages of aplacophorans do form a monophyletic clade sister to Polyplacophora (chitons), a grouping called Aculifera (Scheltema 1993).

Support for the Aculifera hypothesis is important for interpretation of plesiomorphic characteristics of Mollusca (Kocot *et al.* 2011) but many questions remain unanswered. No *bona fide* aplacophoran fossils are known (but see Sutton *et al.*, 2012 and references therein) and only one cladistic morphological analysis has addressed solenogaster phylogeny (Salvini-Plawen 2003; see below). Although aplacophorans are not the basal-most molluscs as previously thought, understanding their evolutionary history is critical to interpretations of early molluscan evolution and will help us polarise key morphological characters for Aplacophora, Aculifera, and Mollusca as a whole.

Within Caudofoveata, three or four families are recognise (reviewed by Todt 2008). The larger taxon Solenogastres is divided into more than 25 families in four orders (reviewed by García-Álvarez and Salvini-Plawen 2007). However, cladistic morphological analyses by Salvini-Plawen (2003) generally failed to recover most orders and several families as monophyletic, suggesting that the existing taxonomy does not reflect the evolutionary history of the group or a lack of phylogenetic signal in the analyzed data. Salvini-Plawen (2003) stated "...the available [morphological] characters are extremely homoplastic and that homology decisions are far too uncertain to accept the resulting trees as reflections of the phylogeny of the Solenogastres." Therefore, molecular data provide an excellent alternative source of characters to evaluate aplacophoran phylogeny and evolution.

Unfortunately, solenogasters have been shown to pose significant challenges to molecular systematists (Okusu and Giribet 2003; Meyer *et al.* 2010; Kocot unpublished data). All authentic nuclear rRNA gene sequences obtained to date contain highly GC-rich regions with highly stable secondary structures. Because of this, attempts to amplify rRNA genes from solenogasters often fail, yield sequences from prey, or yield a chimaeric amplicon with fused pieces from both the solenogaster and its prey. Attempts to circumvent this problem with mitochondrial (mt) markers resulted in poorly resolved topologies (Kocot *et al.*, unpublished data) and designing aplacophoran-specific primers for nuclear-protein coding genes proved difficult, time consuming, and relatively expensive for the amount of data yielded. Therefore, we elected to employ a phylogenomic approach to reconstruct a phylogenetic framework for Aplacophora. To this end, we collected transcriptome data from ten neomenioids, two chaetoderms, and one chiton and conducted a preliminary phylogenomic analysis based on these plus publicly available aculiferan and outgroup transcriptome and genome data.

METHODS

Briefly, total RNA was extracted from frozen or RNAlater-fixed specimens that were starved prior to fixation when possible. Complimentary DNA (cDNA) libraries were prepared with the SMART cDNA library construction kit (Clontech). Full-length cDNA was then amplified using the Advantage 2 PCR system (Clontech) using the minimum number of PCR cycles possible (usually 15-19) and sent to the HudsonAlpha Institute for Biotechnology (Huntsville, AL, USA) for Illumina TrueSeq library preparation and Illumina 2 X 100 bp paired-end sequencing.

Raw reads were assembled on the Auburn University Molette Lab SkyNet server using Trinity (Grabherr et al., 2011) with the default settings. Sanger and 454 transcriptome data were processed and assembled using the EST2uni pipeline (Forment et al., 2008) following the approach used by Kocot et al. (2011). TransDecoder (https://sourceforge.net/p/transdecoder/) was employed to translate contigs to amino acids. In order to exclude exogenous contamination in our transcriptomes, we employed a BLAST-based filter prior to orthology inference followed by manual evaluation of amino acid alignments and single-gene trees. Orthology inference and alignment curation generally followed the methods of Kocot *et al.* (2011).

Phylogenetic analyses were conducted using ML in RAxML 7.3.8 (Stamatakis, 2006) and BI in PhyloBayes MPI 1.2f (Lartillot et al., 2009). For the ML analyses in RAxML the PROTCATLGF model was used and topological robustness (i.e., nodal support) was assessed with 100 replicates of nonparametric bootstrapping. For the BI analysis in Phylobayes, the options "-dir" and "-gtr" were employed to account for site-specific rate heterogeneity and use a general time reversal model of amino acid evolution (Lartillot and Philippe, 2004).

PRELIMINARY RESULTS AND DISCUSSION

We identified 193 genes that showed no evidence of paralogy. As few as eight taxa were sampled for some genes but, on average, fifteen taxa were sampled per gene. The final data matrix was 56,476 amino acid positions in length with 46.56% missing data, on par with other recent phylogenomic studies (e.g., Kocot et al. 2011, Smith et al. 2011). The resulting topology (Figure 1) was, for the most part, strongly supported with most nodes supported by bootstrap values of 100.

Within Neomeniomorpha, the traditionally recognized order Cavibelonia was not recovered monophyletic. All analyses placed Alexandromenia (Amphimeniidae) as the basal-most neomenioid sampled with strong support (100/100) whereas the remaining taxa traditionally ascribed to Cavibelonia plus the two representatives of Neomeniamorpha formed a well-supported clade (100/100) sister to Pholidoskepia. Hypothesis testing using the SH-test (data not shown) showed that monophyly of Cavibelonia including Alexandromenia is significantly less likely than the topology of the best tree recovered by ML. Pholidoskepia (Meiomenia, Wirenia, Macellomenia, Helluoherpia, and Micromenia) was monophyletic with strong support (100/100). Neomeniamorpha was recovered monophyletic with strong support (100/100) but was admittedly only sampled for two species of Neomenia. Interestingly, Neomeniamorpha was placed within Cavibelonia (excluding Alexandromenia) sister to Proneomeniidae + Simrothiellidae but this placement was weakly supported (56/91). Despite weak support, hypothesis testing based on the ML topology rejected placement of Neomeniomorpha sister to the clade including all of the traditional cavibelonians except Alexandromenia. The apparently non-monophyletic taxon "Cavibelonia" was defined on the basis of the presence of hollow sclerites (Salvini-Plawen 1978). Our results suggest multiple independent evolutionary origins of hollow sclerites.

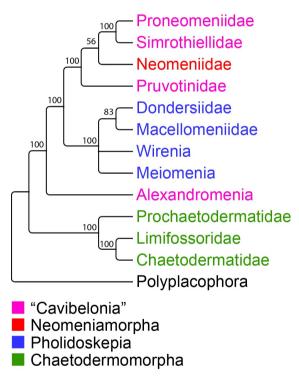


Fig.1. Summary cladogram showing inferred relationships among sampled families of Aplacophora. Bootstrap support values are listed at each node. Nodes with support values <50 were collapsed.

This preliminary molecular phylogeny of Aplacophora drastically differs from the morphology-based taxonomy of this interesting but often overlooked group. Molecular phylogenetics practically turns upside-down previous hypotheses of phylogenetic relationships in both Neomeniomorpha (a cavibelonian taxon as the first branch within the clade) and Chaetodermomorpha (Prochaetodermatidae as the first branch within the clade).

Although this work represents a significant advance in understanding of the phylogeny and evolution of Aplacophora, representatives of several taxa have not yet been sampled. Fortunately, during recent research cruises in Antarctica and in the Pacific, we have collected numerous specimens of aplacophorans including representatives of several key taxa not yet sampled (e.g., a representative of the solenogaster order Sterrofustia). Future directions for this work include using transcriptome data to design probes for a target capture approach (e.g., Lemmon and Lemmon 2012) that could be used to "mine" gene fragments of interest from taxa from which only genomic DNA (even low-quality gDNA such as that of very old museum specimens) and not RNA is available.

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Snail water feature in Brouchart Gardens, Vancouver Island, British Columbia. 2013

Patella shells from the Canary Islands as seasonal retrospective temperature archives

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ABSTRACT



The aboriginal people from the Canary Islands collected, consumed and accumulated over time marine mollusks (primarily Patellidae). Prehistoric *Patella* shells may be used as paleoenvironmental proxies because the oxygen isotopic composition (δ^{18} O) of the shell is mainly influenced by temperature and seawater δ^{18} O values during calcification. To evaluate the potential of *Patella* species from the Canary Islands as environmental sentinels, live specimens of *Patella*, along with sea surface temperature and water samples were collected regularly for eight months in Tenerife Island. Shell margin δ^{18} O values, which depict the oldest growth episode, varied from +1.9‰ in March to +0.1‰ in September. Shell margin δ^{18} O values correlated negatively with observed temperature (p<0.001) whereas seawater δ^{18} O values did not vary seasonally. Calculated temperatures using the equation by O'Neil *et al.* (1969) were underestimated by 3.3±0.7°C. If shell margin δ^{18} O values are corrected by a factor of 0.72‰, then calculated and observed temperatures overlap. Conclusively, the δ^{18} O values of fossil *Patella* shells from the Canary Islands should be corrected by subtracting 0.72‰ before paleotemperature inferences. On-going research includes the study of *Patella* specimens recovered from shell middens and paleontological sites (1) to deduce paleotemperatures before (Pleistocene-early Holocene), during (late Holocene) and after (from the 15th century to the present) prehistoric times, and (2) to infer variations in season of shellfish collection during aboriginal occupation in the archipelago.

INTRODUCTION

The Canary Islands are a low latitude (27-29°N) oceanic and volcanic archipelago located only ~95 km off from Moroccan coast (Fig. 1). These islands were first time inhabited by ancient Berber people from Northwest Africa (Maca-Meyer et al. 2004) about 2,500 cal. years BP (Navarro-Mederos 1983). The indigenous people (locally called the Guanches) followed a sedentary socioeconomic lifestyle based on cattle and agriculture without metals. Since they were surrounded by ocean, resources, (mostly shellfish), were an important part of their subsistence strategy (Mesa-Hernández 2007). Thus, these islands contain numerous humaninduced late Holocene shell middens, which were exploited by the aborigines since the 1st millennium BC until the end of the 15th century, when Spaniards conquered these islands (Fig. 1). Shell middens are dominated by rocky intertidal species, especially limpets of Patella genus (Mesa-Hernández 2007). These marine gastropods are potentially valuable high-resolution retrospective environmental proxies during prehistoric times. Mollusk shells grow by periodic accretion of calcium carbonate and constantly record fluctuations of the environment. Most species precipitate their shells in oxygen isotopic equilibrium with the ambient water. The δ^{18} O values of the shell mainly reflect the combined effects of sea surface temperature and the δ^{18} O values of the water (Epstein et al. 1953, Grossman and Ku 1986). This relationship is mathematically expressed by empirically derived equations for both calcite and aragonite. As water temperature varies through seasons, shifts in the shell δ^{18} O values should reflect variations in seasonal temperatures if seawater δ^{18} O values remain stable year round. Accordingly, shell δ^{18} O values may be used as seasonal paleotemperature proxies. However, some species seem to be affected by somewhat vital effect and as a result, oxygen isotopic offsets may occur between observed and expected shell δ^{18} O values assuming isotopic equilibrium (Fenger *et al.* 2007). Accordingly, if target species have not been previously investigated, a detailed proxy calibration using modern analogs is essential prior to any paleoenvironmental inferences. The goal of this research was to conduct a detailed calibration of the two dominant Patella species from the Canary Islands using living specimens and local environmental data collected jointly. This study represents the first detailed modern isotopic baseline for coastal ecosystems in the Canary Islands.

MATERIALS AND METHODS

Samples were collected every 15 days from July 2011 to April 2012 in the Puertito de Güímar locality (Latitude: 28°17'02"N; Longitude: 16°22'33"W), SE Tenerife, Canary Islands (Fig. 1A). Several specimens of the Patellidae limpet Patella tenuis crenata d'Orbigny, 1840 and Patella ulyssiponensis aspera Roding, 1798 were live-collected, along with coastal seawater samples and sea surface temperature measurements. These species were selected because they are the most abundant in the study site as well as in local archeological shell middens and are also preserved in Pleistocene and pre-human Holocene raised marine terraces. Both species overlapped in distribution within the intertidal rocky zone. All analyses were conducted in the Stable Isotope Facility of the Earth and Environmental Sciences Department of the University of Kentucky. Three limpet shells from each sampling date were selected for oxygen stable isotope analyses of the shell margin (=last growth episode closest to death). Shells were carefully cleaned using DI water and ultrasonication. About 150 µg of carbonate was obtained from each specimen by passing slowly a dremel drill with a 1mm bit by hand. A total of 96 isotopic analyses were acquired from Patella shells. Carbonate powder was placed in a 6 ml ExetainerTM vial, flushed with helium, and converted to CO₂ gas by adding 0.1 ml of 100% H₃PO₄ at 25°C during 24 hours. The CO₂ gas was measured using the GasBench II connected to the Finnigan Delta PLUS XP isotope ratio mass spectrometer (IRMS). A total of 17 seawater samples collected the same days as limpets were measured in a Picarro device for stable oxygen isotope values. Stable isotope results are reported in δ notation relative to the international standard PDB for carbonates and SMOW for seawaters. Analytical reproducibility was better than 0.1% based on the repeated measurements of the international standards. A single shell was sampled five times at the shell margin and the reproducibility was better than 0.1% as well.

Simple linear regression and Pearson correlation analyses were conducted using *Past 2.18b* software to evaluate the potential relationship between variables. The calcite-water paleotemperature polynomial equation by O'Neil *et al.* (1969) was selected to calculate shell δ^{18} O values using observed environmental variables and assuming isotopic equilibrium:

$$SST(^{\circ}C) = 16.9 - 4.38(\delta c - \delta w) + 0.1(\delta c - \delta w)^{2}$$

Seawater δ^{18} O values were transformed from SMOW to a PDB scale by subtracting 0.27% (Ferguson *et al.* 2011). Other pale-otemperature equations for the calcite-water system available in the published literature yielded similar results.

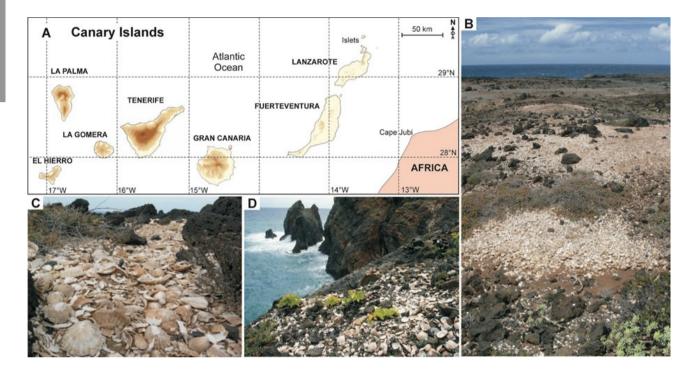


Fig. 1. Map of the Canary Islands (A). Field photographs of late Holocene shell middens from Teno Bajo, Tenerife Island (B-C) and Vallehermoso, La Gomera Island (D). Photographs are courtesy of Eduardo Mesa (Universidad de La Laguna).

RESULTS AND DISCUSSION

Sea surface temperatures (n=17) of coastal waters from SE Tenerife Island varied from 18.1°C in February to 23.0°C in September. About 5°C of seasonal variation in temperature was documented in the study site. In contrast, seawater $\delta^{18}O$ values varied minimally through seasons (<0.4‰) and showed an average annual value of +0.8±0.1‰ (n=17). Such seawater $\delta^{18}O$ value was adopted for subsequent paleotemperature calculations.

Patella shell δ^{18} O values varied as much as 1.8% along 8 months. Shell margin δ^{18} O values (n=96), which depict the conditions closest to the organism's death, varied from +1.9% in March to +0.1% in September (Fig. 2A). The average standard deviation of shell δ^{18} O values per sampling date among individuals was 0.1%. Shell-edge δ^{18} O values correlated negatively with sea surface temperatures (Fig. 2B) whereas no correlation between water and shell δ^{18} O values was observed. Calculated sea surface temperatures using the equation by O'Neil *et al.* (1969) and the measured shell and seawater δ^{18} O values were about 3.3±0.7°C cooler than observed local temperatures. Such offset was constant through seasons (Fig. 3A). The underestimation of temperatures can however be rectified if shell δ^{18} O values are corrected by subtracting 0.72% (Fig. 3B). Previous studies on other *Patella* species from other locales have also documented a similar magnitude of isotopic offset between measured and predicted shell δ^{18} O values, varying from 0.7% to 1.0% (Shackleton 1973, Cohen and Tyson 1995, Fenger *et al.* 2007, Ferguson *et al.* 2011). This offset has been interpreted as a vital effect or biological fractionation. However, because the isotopic offset is constant and predictable, a correction can be applied to measured shell δ^{18} O values.

This study contains notable archeological and paleontological implications. Thus, when studying archeological and fossil *Patella* shells from the Canary Islands, a subtraction of 0.72‰ should be applied to measured shell δ^{18} O values in order to obtain accurate temperature inferences using O'Neil et al. (1969) paleotemperature equation. This work emphasizes the importance of studying local modern analogs of target species before using fossils for paleoenvironmental reconstruction. The results here and published work by others suggest that *Patella* specimens are likely to exhibit a constant positive isotopic offset from the expected isotopic equilibrium ranging from 0.7‰ to 1‰, regardless of latitude and species. The magnitude of such offset may vary slightly with the adopted seawater δ^{18} O value and the paleotemperature equation used.

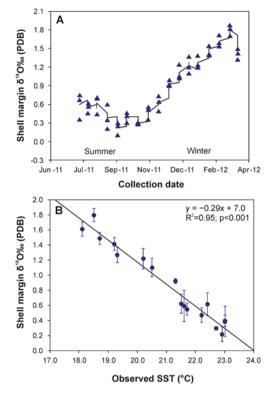


Fig. 2. Stable oxygen isotope results of live-collected *Patella* shells from Tenerife, Canary Islands. (A) Shell margin oxygen isotope values of *Patella* through seasons. Note that three adult limpets were analyzed per sampling date. (B) Relationship between the shell margin oxygen isotope values of *Patella* and observed sea surface temperature at the time of limpet collection.

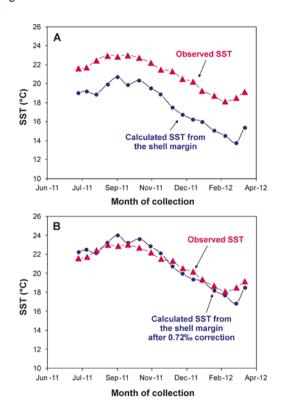


Fig. 3. Calculated sea surface temperatures from the shell margin through seasons. (A) Comparison between calculated and observed SST before vital effect correction. (B) Comparison between calculated and observed SST after vital effect correction

CONCLUSIONS

This study illustrates that Patella precipitates shell material throughout all seasons in the Canary Islands and that shell-edge δ^{18} O values track credibly the sea surface temperature closest to the organism's death. However, shell δ^{18} O values show a constant positive offset from isotopic equilibrium of 0.72%. This offset is interpreted as a vital effect and is consistent with previous Patella studies from higher latitude regions. Accordingly, measured shell δ^{18} O values should be corrected by subtracting 0.72% before local temperatures can be predicted. Pending work with modern samples includes (1) a detailed shell microstructure study of Patella, and (2) intra-shell stable oxygen isotope analyses along shell growth direction. Such analyses will be used to evaluate shell growth patterns and environmental seasonality along Patella's lifespan. Finally, fossil shells prior to human occupation (>2,500 cal yr BP) and archeological shells during aboriginal settlement (from 2,500 cal yr BP to the 15th century), will be studied isotopically to infer potential variations in seasonal paleotemperatures before and after African Berber people occupied the archipelago. Such data may in turn be used to evaluate potential relationships between variations in human subsistence strategies and the environment.

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The significance of byssi and their morphological diversity within the superfamily Pterioidea

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INTRODUCTION

Many adult bivalve molluscs attach to different kinds of substrata by the byssus. The morphology of the byssus can significantly vary within this class of molluscs and plays a very important role in their survival at the bottom. Also, byssus has a historical significance in everyday life of southern areas of Italy and Portugal, where byssi of Pinna nobilis were collected for the production of "marine silk" from ancient times until now. Sardinia and Puglia were the main centers for the processing of extremely fine golden "marine silk" that was used for the production of a very light and warm knitwear, such as gloves, hats, socks, berets, ties, also decorative fabrics, and tapestries. Chiara Vigo from Sardinia is one of the few women, who are still manufacturing byssal cloth today. The Ethnographic Museum of Antioquia in Sardinia, Italy, and approximately ten other museums around the world house less than 100 examples of cloth of the legendary sea silk and other related artworks (Fig. A-C). It is interesting to note that as the Latinized zoological term "byssus" was derived from the Greek word "bussus" that signified fine sea silk (Feen, 1949). As a rule, textiles woven from byssal threads were highly praised in all ancient cultures where sea silk was known. For example, byssal threads were identified in a Roman Centurion's cloak, the raiment of King Tutankhamen, and might have been the material of the Golden Fleece sought by the mythical Jason and the Argonauts.

At the present, the morphology of the byssus has been studied most extensively in the family Mytilidae (e.g., Price 1983, Vekhova 2007). There is relatively little information on morphology of the byssus and its chemical composition for some Pinnidae (*Pinna nobilis, Atrina rigida*) in a number reports (Jackson *et al.* 1953, Pearce & LaBarbera 2009). The morphology of the byssus has been previously briefly described for some Pterioidea, pearl oysters and their relatives (Banu et al., 1980; Tëmkin, 2006a, b). To date, there are no studies addressing the connection between the morphological structure of the byssus and an ecological diversification for any bivalve lineage. The aim of this investigation was to elucidate of the morphological structure of byssus within the superfamily Pterioidea across all the genera in relation to its significance in ecological diversification.

METHODS

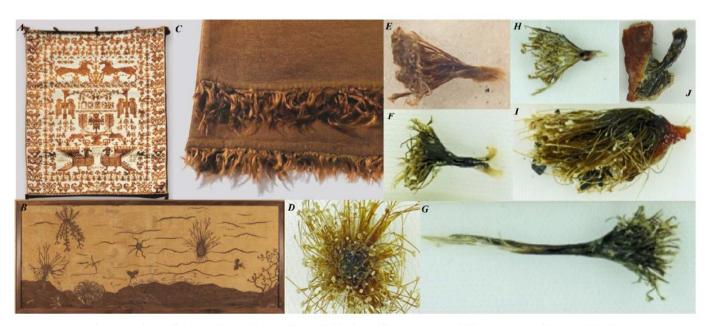
The byssi were isolated and its morphology examined using light and scanning electron microscopy (SEM). For SEM all samples were prepared as described (Vekhova, 2007). Dried samples were mounted on aluminum stubs, sputter-coated with gold-palladium using a Cressington Sputter Coater 108 Auto, and then viewed on a Philips XL-30 ESEM scanning electron microscope. Specimens were photographed with Olympus Q-Color 5 camera. The study was conducted in the period of December 12 -January 12 in collaboration with I. Tëmkin at the National Museum of Natural History (Smithsonian Institution, Washington DC, USA).

RESULTS

Comparative analysis using light microscopy showed that the investigated species have differences in byssus structure, size and colour among of pterioidean genera (Table). The byssus consists of three parts in all species: 1) a stack of thin multitudinous parallel lamina, typically pale yellow in colour, embedded deeply in the tissue of the foot and connected by a root with the pedo-byssal retractor muscles; 2) usually a short or implicit stem; and 3) a bunch of byssal threads, which can be fused partly or completely in different pterioidean genera with adhesive plaques at the distal end, keeping a mollusc attached to a substratum (Fig. D-J). The microphotographs have shown that in a number species of the superfamily Pterioidea, the shape of adhesive disks presents a brush-like structure differing from that in *Mytilus*, which can be explained by the process of byssus formation in a pedal groove in the molluscs. Also the byssal threads of some Pterioidea species were not covered by a cuticle *completely*, including disk-like forms in *Mytilus*. The marine bivalves of the superfamily Pterioidea, which include four extant families (Pteriidae, Malleidae, Isognomonidae, and Pulvinitidae), lead epifaunal or semiinfaunal life style, attaching by a byssus, although there are abyssate endozoic forms living inside sponges (species of *Vulsella* and *Crenatula*). As with many other bivalves, this group retains the byssus in postjuvenile forms for physical stability on substrata for development of the adult forms (Yonge, 1962).

Some characteristics of the byssus in a range of pterioidean taxa

Species	Length, mm	Color	Byssal threads	Disk
Pinctada imbricata	18	Brown-green	All separated	Brush
Pinctada margaritifera mazatlanica	30	Green stem, yellowish green threads	All separated	Brush
Pinctada capensis	60	Brown stem, green threads	All separated	Brush
Pteria brevialata	21	Brown	Fused completely	Brush
Pteria hirundo	22	Dark brown	Fused partly	Disk
Pteria howensis	12	Light brown	Fused completely	Disk
Pteria loveni	17	Deep brown	Fused completely	Disk
Electroma papilionacea	10	Light yellow	All separated	Brush
Electroma alacorvi	8	Brown stem, green threads	All separated, fused by disks	Brush
Isognomon ephippium	28	Dark green	Fused partly	Disk
Malleus malleus	30	Brown stem, green threads	Fused up to 2/3 length	Disk
Malleus albus	14	Dark brown stem, green-brown threads	Fused up to 2/3 length	Brush
Pulvinites exempla	21	Golden brown	All separated	Disk



Some practice application and natural view of byssus in bivalve molluscs among pterioidean genera: A - Tapestry of Benito Mussolini, B - "Marine silk" in visual art, C - shawl made from byssal threads of Pinna nobilis, D - byssus of Pulvinites exempla, E - Electroma alacorvi, F - Malleus albus, G - Malleus malleus, H - Pteria hirungo, I - Pinctada capensis, J - Pteria brevialata.

The greatest variation of byssus morphology was observed in the family of Pteriidae (Fig. E, H-J). In particularly, the byssus structure is developed in marine pearl oysters of the genera *Pinctada* and *Pteria*. Species of *Electroma* have a small byssus with the fine numerous filaments of adhesive disks which attach to hard corals. In *Pulvinites exempla* (family Pulvinitidae) the byssus is adapted for attachment to hard substrata. For survival in soft sediment and in mangrove ecosystems, *Malleus albus* and *Isognomon ephippium* use a brush-like byssus.

FUTURE WORK

It would be good to conduct comparative study of the morphology pedal groove and glands located there. Obviously molluscs of the genera *Mytilus*, *Dreissena* and *Pinna* have different byssi, which are not homologues. The data reported here concerning byssus gland morphology among the pterioidean genera will help us to expand knowledge about the process of byssus formation in bivalve molluscs and can be used for anatomical atlases and textbooks in zoology.

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Identifying the molecular link between photosensitive tissues and eyes of the bay scallop, Argopecten irradians.

Autum N. Pairett

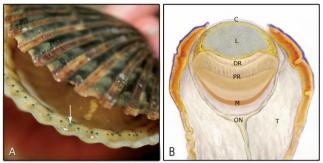
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Molluscs utilize a vast array of photoreceptive structures. Whether they are complex eyes capable of spatial vision or simple photoreceptive neurons found in the mantle tissue (extra-ocular photoreception, EOP), photoreceptive structures provide environmental information that can direct foraging, habitat selection, reproduction, and predator avoidance behaviors. EOP is ubiquitous in molluscs, while spatial vision has evolved independently multiple times (von Salvini-Plawen 2008). This pattern suggests that, through the recruitment of photosensitive machinery from one system to another, EOP may be an evolutionary source of eyes. Studying the molecular relationship between eyes and EOP can provide evidence for the mechanisms involved in eye evolution in molluscs (such as gene co-option). To date, only the eye and its photoreceptive protein, opsin, has been compared at the molecular level to EOP of the cuttlefish (Mäthger *et al.* 2010). Other independently derived molluscan eyes, as well as the downstream molecules of the phototransduction pathway (which converts light from the environment into a chemical signal), have yet to be examined in this context. Expanding the comparison of the molecular components in EOP and eyes will be important to further our understanding of eye evolution in molluscs.

Scallops (Pectinidae), a diverse family of marine bivalves, represent an excellent opportunity to study the relationship of EOP and vision. Scallops are well known for their unique mirror-type eyes found along the mantle edge on modified tentacles. The scallop eye is composed of a cornea, lens, two retinas (proximal and distal), and a mirror lining the back of the eye (Figure 1). The mirror reflects and focuses light back onto one of the two retinas found in the middle of the eye. Interestingly, each retina utilizes a different molecular pathway for phototransduction, as evidenced by the different opsins expressed in each retina (Gq-opsin in the proximal retina and Go-opsin in the distal retina; Kojima *et al.* 1997). While the anatomy and physiology of the scallop eye has been studied extensively, much less is known about the molecular components of scallop vision. In contrast, virtually nothing is known about EOP in scallops. While it is widely accepted that bivalves have photosensitive neurons in their mantle, this has been explicitly examined in only a few species (e.g. Kennedy 1960). One way to test whether mantle tissue is involved in EOP is to determine 1) if genes involved in phototransduction are expressed in that tissue and 2) whether expression of these gene changes in relation to the presence or absence of light.

Because it has been demonstrated that phototransduction genes do not arise *de novo* (Plachetzki et al. 2010), I tested whether the scallop phototransduction systems in the eye are shared with EOP in the mantle. To do so, I analyzed gene expression in visual (eyes), putatively photoreceptive (tentacle), and non-photoreceptive (adductor muscle) tissues from the bay scallop, *Argopecten irradians*. Two hypotheses were tested in this study: 1) If scallop mantle functions in EOP and are not solely used for chemoreception, it will contain the molecular components of the phototransduction pathway, which are required for photoreception; 2) Photosensitive organs will show increased expression levels of phototransduction genes under light conditions when compared to the dark treatment. The results from this work represent the first molecular characterization of mantle-based EOP in bivalves and begin to identify links between two independent photoreceptive systems that, with further work, may implicate mechanisms that generated the diversity of molluscan eyes.

Fig. 1. The mirror type eye of the scallop *Argopecten irradians*. (A) Scallops have up to 200 single chambered eyes along their mantle edge, indicated by the white arrow. (B) The eye sits on a tentacle (= T) and the major internal structures of the scallop eye are indicated. C= cornea, L= lens, DR= distal retina, PR= proximal retina, M= mirror, ON=optic nerve. Figure from Pairett and Serb 2013.



MATERIALS AND METHODS

To target gene expression patterns related to photoreception, I implemented an experimental design meant to maximize the expression of photoreception related genes (Figure 2). Specifically, three tissues were collected from *A. irradians* individuals under two separate light treatments: 1) nine hours in light

and 2) nine hours in dark (Dalal et al. 2003, Halstenberg et al. 2005). These tissues included the eyes, mantle, and adductor muscle. Tissues were collected immediately after each treatment, then flash frozen or stored in RNAlater for future RNA extraction. Dark treatment tissues were collected under red light. RNA was extracted using the

Ambion Ribopure kit (cat. # AM1924) and sequenced using the paired-end protocol for the Illumina HiSeq2000 at the Iowa State University DNA Facility. Completed sequence reads were first digitally normalized to remove any redundant reads, followed by assembly, open reading frame (ORF) prediction, and gene expression analysis using the Trinity package (Haas *et al.* 2013). All sequence reads were assembled together to create a reference dataset of genes expressed in *A. irradians* tissues. Sequences from each tissue and treatment were then mapped against the completed *A. irradians* eye transcriptome to calculate gene expression levels.

To identify key genes from the phototransduction pathway and the circadian clock within the *A. irradians* transcriptome assembly, I took a targeted BLAST approach. Protein sequences for the genes from each pathway were downloaded from NCBI, then blasted against the scallop transcriptome in the program Geneious (v. 5.5, Biomatters), as done in Pairett and Serb (2013). Reciprocal blasts were used to confirm the identity of each gene.

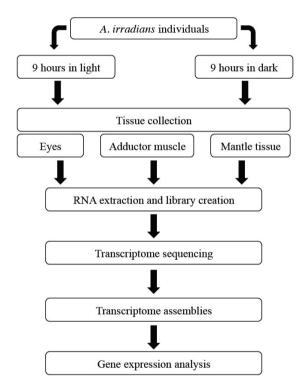


Figure 2. Flowchart of experimental design.

RESULTS AND DISCUSSION

The Illumina HiSeq2000 sequencing run resulted in nearly 1.5 trillion 100 bp long reads (1,490,752,848 reads), with each of the six libraries averaging about 124 million reads. Assembly of all six libraries resulted in over 230,000 transcripts, which Trinity (Haas et al. 2013) grouped into just over 176,000 components (or genes). After annotating the assembled transcriptome, I found that 37 of the 38 photoreception related genes queried are present in the scallop tissues. These genes include the Gq-phototransduction pathway, Gi-photransduction pathway (which includes components from both the Go- and Gt-pathway), and circadian rhythm. When expression of the phototransduction and circadian rhythm genes was examined in each tissue individually, I found that all three tissues (eyes, mantle, and adductor muscle) expressed some of the genes that are important for photoreception. Specifically, the eye and mantle contain components from all photoreception pathways. The mantle tissue did not express the Go-opsin. This pattern supports the prediction that the mantle tissue is photoreceptive and implicates the Gq-phototransduction pathway in the putative photoreceptive ability of the mantle <cont>

tissue. The second phototransduction system known to be used in scallop vision, the Go-phototransduction pathway, is probably restricted to the eye. The adductor muscle was also found to express some of the components from each of the photoreceptive pathways, but importantly, no opsins (the photoreceptive protein required for vision in nearly all animals) were found in the adductor muscle. This result supports the prediction that the adductor muscle is not photoreceptive.

A pairwise analysis of differential gene expression between each tissue and treatment has found a large number of differentially expressed genes in each case (for three examples, see Figure 3). Because of my interest in the photosensory system of the eye and mantle, I specifically examined the differential gene expression of the three photoreceptive pathways. When comparing the eye under light and dark conditions, I found that opsins are upregulated in light. This pattern fits with the previous work in *Limulus* (Dalal *et al.* 2003) and fish (Halstenberg *et al.* 2005) that showed opsin expression peaks nine hours after sunrise. Interestingly, when comparing the mantle tissue in light and dark, only components from circadian rhythm were found to be upregulated in dark. Finally, when comparing eye and mantle under light conditions, I found that components of the Gq-phototransduction pathway were upregulated in the eye compared to the mantle tissue. I also found several opsins upregulated in the eye compared to mantle, including the Go-opsin and the two copies of Gq-opsin identified in my lab previously (Serb *et al.* 2013). Most interestingly, seven additional opsin sequences were found to be upregulated in the eye compared to the mantle. This result shows the level of previously undescribed molecular diversity in the scallop eye and highlights the need for additional work on this system.

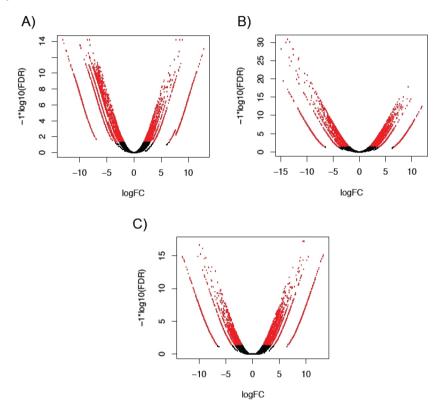


Fig. 3. Volcano plots showing differential gene expression between tissues and light conditions. The x-axis represents fold change in expression while the y-axis shows the negative false discovery rate (FDR) on a \log_{10} scale. Red dots represent differentially expressed genes, while black dots represent genes that are not significantly differentially expressed. A= eye in dark versus eye in light, B= eye in light versus mantle in light, C= mantle in dark versus mantle in light.

CONCLUSIONS AND FUTURE WORK

In this study, I have sequenced the largest molecular dataset of photoreceptive structures in the scallop. These data will be important for future work identifying genes important to photoreception or other sensory systems, such as transmembrane protein receptors. Within this dataset, I have found evidence that scallop mantle tissue is photoreceptive and probably uses the same Gq-phototransduction system that is found in the proximal retina of the scallop eye. The Go-phototransduction system, from the distal retina, appears to be restricted to the eye, as Go-opsin was not found in mantle tissue. Additionally, I have identified at least seven new putative opsins that are expressed in the scallop eye and mantle. Future work will focus on characterizing these opsins to and examining the evolutionary processes involved in their diversification within scallops. Further work identifying the differentially expressed genes between the scallop eye, mantle, and adductor muscle will also be completed to find other genes of interest that may be important to the function of each tissue.

ACKNOWLEDGMENTS

I would like to thank the Malacological Society of London for their support of this project. The Conchologists of America, Sigma Xi, and the Iowa Academy of Science also provided funding for this work. I also thank members of the Sanibel-Captiva Conservation Foundation Marine Laboratory, especially Eric Milbrandt and Richard Bartleson, for their help and support inconducting my experiment. Srihari Radhakrishnan assisted with the sequence assembly. Brad Fleming assisted with tissue collection and analyses.

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OBITUARY

Richard Kilburn

It is with great sadness that I have to report the passing of Dr Richard N. Kilburn – 'Dick' to all his many friends and correspondents. He died in the early morning of July 26, 2013, while many of us were at the World Congress of Malacology. His sudden death after a short illness will represent a shock to many of us and certainly a loss to malacology, particularly to those working with 'turrids'.

His knowledge of the southern African and Indo-West Pacific malacofauna was exceptional. His many papers will bear witness to his dedication and to his contribution to malacology. He will be missed, both for his malacological knowledge and his sense of humour.

Dai Herbert

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(originally posted on the Mollusca listserver)



Sexual selection in marine snails using littorinids as model species

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Terence receiving the Annual Award certificate from Prof. Mark Davies (on behalf of the President and Council of the Malacological Society of London) during fieldwork in a mangrove in Hong Kong.



Sexual selection has been intensively studied in birds, mammals and insects. In particular, many major theories and empirical studies have their origins in studies of mating systems in insects, the largest animal class. The second most species-rich class after the insects, the Gastropoda, has however received little attention in past decades, but has recently been subject to increasing interest in the context of sexual selection studies. The delay of malacologists to embrace sexual selection theory in gastropods has been suggested to be because many gastropods are hermaphroditic, and hence sexual selection theory has been thought to unlikely to apply to these species. Darwin himself stated that gastropods, as "lower animals", lacked the sensory and mental capacity to choose mates, commenting that these animals had "too imperfect senses and much too low mental powers to feel mutual rivalry, or to appreciate each other's beauty or other attractions".

Recent studies on hermaphroditic land snails and sea slugs have, however, suggested the occurrence of sexual selection in the Mollusca. In fact, most marine snails are dioecious, but only two families (Littorinidae and Buccinidae) have been examined in the context of sexual selection. Using two mangrove snails, *Littoraria ardouiniana* (HEUDE 1885) and *L. melanostoma* (GRAY 1839) (Littorinidae) as model species, my PhD thesis documented the occurrence of sexual selection in various mating stages of these marine snails. This has yielded new insights into the modes of operation of sexual selection in animals.

How can males find females in the dense mangrove tree canopy?

Understanding the mating behaviour of the two littorinid snails was a prerequisite for studies of sexual selection. Mate location in the dense mangrove tree canopy would appear to be extremely difficult (Figure 1) and may require some form of adaptive strategy for the two littorinids to achieve reproductive success. To investigate this, mate location through mucus trail following was investigated in experimental arenas under controlled laboratory conditions (Figure 2). The intensity of trail following was compared between different sex combinations in both the mating and the non-mating seasons. I found that males of both species were able to recognize conspecific females from their mucus trails (they followed mainly trails laid by conspecific females, but not conspecific males or heterospecifics) in the mating season, but not in the non-mating season (Ng et al. 2011; a video of trail-following behaviour in L. ardouinina is available at the Science news forum via the following link: http://news.sciencemag.org/sciencenow/2011/07/video-oozing-toward-love.html?ref=hp) and hence that trail following is likely to increase reproductive success in these littorinids. These data also allowed the subsequent formulation of sexual selection hypotheses (i.e. whether males followed mucus trails laid by 'high'-quality but not 'low'-quality females, see below). Interest in the possible roles and importance of trail following also resulted in a review paper on this subject area with collaborators (Ng et al. 2013).

Competitive and choosy males

Ever since Darwin, conventional wisdom in the field of sexual selection has been that males are 'competitive' and females are 'choosy'. Increasing evidence has, however, shown that males can also be choosy and male mate choice is actually common among animals. Interestingly, males of the mangrove littorinids can be both competitive and choosy! By allowing snails to crawl on experimental arenas (Fig. 2) and mate in glass chambers (Figure 3), I investigated males' preferences for female size during the three consecutive mating steps (trail following, shell mounting and copulation) and whether two males 'fought' for females when they encountered a female at the same time. I found that males of *L. ardouiniana* were more choosy and aggressive than males of *L. melanostoma* in finding their mates.

Fig.1. A mating pair of Littoraria ardouiniana in the dense tree canopy of Kandelia obovata at Tsim Bei Tsui mangrove, Hong Kong



Male L. ardouiniana preferred to follow and mate with large females and they mated with large females for longer durations than with small females, but such preference was not found in males of L. melanostoma. Female L. melanostoma did, however, frequently reject males by pushing away the penes using their snouts. Male L. ardouiniana aggressively pushed against each other when they encountered another male on a female and large males always won these 'mating battles', but males of L. melanostoma rarely 'fought' for females. These differences between the two species can be attributed to the degree of variation in female quality (female fecundity increased dramatically with body size in L. ardouiniana but not L. melanostoma, Ng & Williams 2012) and mate availability (the population sex ratio was male-biased in L. ardouiniana but had a balanced sex ratio in L. melanostoma). These results demonstrate that sexual selection may operate in various ways and can be complex (e.g. cooccurrence of male mate choice and male-male competition) in these mangrove snails.



Size-dependent male mate preference and its contribution to size-assortative mating

Size-assortative mating (a positive correlation in body size between male and female mating partners) is a widespread phenomenon in animals, including littorinids. A traditional explanation of size-assortative mating in terms of sexual selection is that all males prefer to mate with large (more fecund) females and large males have a size advantage over small males in obtaining these females. I conducted field surveys and measured sizes of mating pairs of snails in the mangrove, and found size-assortative mating in the two littorinids species. I therefore examined whether this mating pattern is driven by male mate choice in these snails. In the laboratory, I tested whether or not male mate choice occurred in each of the three consecutive mating steps, by allowing snails to crawl on experimental arenas and mate in glass chambers (as above). Male mate preference in *L. ardouiniana* was size-dependent (i.e. large males preferred large females, but small males were less selective, Ng & Williams under revision), which demonstrates an alternative mechanism to the traditional explanation of sexual selection in driving size-assortative mating.

Littorinids as a model system to study sexual selection in marine snails

Since mating behaviour in dioecious marine snails displays common characteristics and is relatively simple, involving little complex courtship behaviour compared to land snails, sexual selection in littorinids can be used as a template to interpret strategies in other dioecious species. Here I summarize all possible sexual selection scenarios in littorinids based on my thesis and literature review (Fig. 4). This model should be useful for other malacologists who are interested in sexual selection in marine snails. In brief, sexual selection may take place in the forms of male mate choice and male-male competition during the three mating steps as

demonstrated in the model (Fig. 4). The rejection behaviour of females may be related to sexual conflict or female mate choice, but further study is needed to clarify which is likely to be more important. Other studies have also shown that females of some species may mask their gender identity in their mucus trails due to costs related to mating, suggesting sexual conflict. An additional note is that female snails, like many insects, may store sperm, but very little is known about postmating (or prezygotic) sexual selection via sperm competition and cryptic female choice in littorinids, so this remains a fascinating area to explore further.

Fig. 3. A: a small male *L. ardouiniana* copulating with a large female in an experimental glass chamber; B: two male *L. ardouiniana* 'fighting' on top of a female

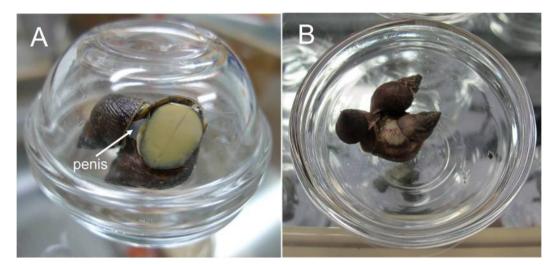
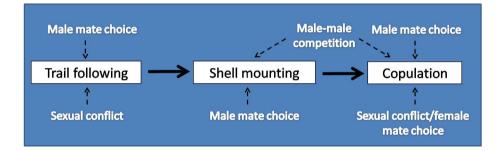


Fig. 4. A theoretical model to illustrate all possible sexual selection scenarios (mate choice; competition and sexual conflict) throughout the mating process (boxes, trail following to locate a mate; shell mounting and copulation) in littorinid snails.



ACKNOWLEDGEMENTS

"If I have seen further, it is by standing on the shoulders of giants." I quote this classic phrase from Isaac Newton, not to claim that I am a great scientist but to express my sincere gratitude to all the 'giants' that inspired, advised and encouraged me throughout the pursuit of my PhD degree. This work would not have made possible without help from the following people. Foremost, I would like to thank my primary supervisor Prof. Gray A. Williams for his guidance, encouragement and inspiration to my interests in science and in ecology. I am also extremely grateful to my colleagues from the Swire Institute of Marine Science (SWIMS), especially Dr V. Thiyagarajan (my co-supervisor), Dr Priscilla T. Y. Leung, Dr Ng Wai Chuen, Dr Stephen Cartwright, Dr June Leung, Cecily Law, Kelvin Wong and Andy Yi for their valuable advice on my study and help with my laboratory and field work. For constructive ideas and useful discussions on my work, I thank my collaborators and academic friends including Prof. Mark Davies (University of Sunderland), Dr Sara Hintz-Saltin (Göteborg University), Dr Richard Stafford (Bournemouth University), Prof. Kerstin Johannesson (Göteborg University), Prof. Christopher McQuaid (Rhodes University), Dr David Morritt (University of London), Dr David Reid (Natural History Museum, UK), Dr David Marshall (Universiti Brunei Darussalam) and Dr Kitithorn Sanpanich (Burapha University). Especial thanks go to my parents and my girlfriend Miranda Cheng for their endless love, support and care.

It is my great honour to be the winner of the Annual Award of the Malacological Society of London and I hope that this review of my work will stimulate more malacologists to investigate sexual selection in this fascinating group of animals.

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ANNUAL REPORT OF COUNCIL

Annual report of Council for 2012, delivered by the President, Tony Walker

The President thanked Suzanne Williams for organising the meeting on "Species delimitation and chirality: molluscs as model organisms" at The Natural History Museum, London, 17th April 2013, during which the 120th Annual General Meeting of The Society was held

Membership (reported by Richard Cook)

Membership currently stands at 117, including 14 new members who have joined the Society during last year or at the beginning of this year. Two long-standing members retired, including Tony Cook who served on the Council as Web Manager for many years. Our thanks are extended to Tony for his work and help in running the Society over the years. Sadly, as reported in the Society's August 2012 edition of The Malacologist, Tony South passed away last year.

Finance, for the financial year ending 31 December 2011 (reported by Katrin Linse)

The finances of the Malacological Society have been more than satisfactory during 2012 with a gain £28.7K. Our investments – the COIF Investment Fund and the COIF Fixed Interest Fund both have made gains. In 2012 £20k was transferred from the current account to the COIF Fixed Interest Fund to minimise potential loss caused by the economic downturn. However, the profit-share from the publication of the Journal provided the Society with the major proportion of its income. The Editor of the Journal, Dr David Reid, and the assistant editors are to be commended for the hard work involved in the publishing our scientific journal. Once again sales of the digital archives provided over £7,000 of income; future sales might ease as OUP is planning to give free access to the digital archives of their journals and the digital archive will be sold in subject packages from 2013 onwards.

The Society slightly decreased the values of its various Awards in 2012, as fewer travel bursaries were claimed. More funds were used for the research awards compared to 2011 based on scientific quality of the submitted applications and for the travel awards to the Forum as awards were given out on an actual spending basis.

The Society's independent examiner, Annie Owens, examined the Society's accounts following the Charity Commission and AC-CA rules

Meetings

On behalf of The Society Simon Cragg organized a meeting on *Molluscan Life Histories* 'at the Institute of Marine Sciences, University of Portsmouth on 20-21 April 2012. This was also the place for the Society's 119th AGM. The fifteenth annual *Molluscan Forum* was organized by Jonanthan Ablett and the President and held at the Natural History Museum, London on 28 November 2012: 19 papers and 14 posters were presented from both UK and overseas participants. The Society provided lunch for all attendees and this served to create a cohesive meeting, with excellent opportunity to discuss the posters. The Forum was again held consecutively with the Young Systematists' Forum, affording an opportunity for students to attend both meetings.

Publications

The Malacologist (reported by Georges Dussart)

The Malacologist is circulated to members as an emailed .pdf attachment. However, there are always problems with a small number of members who have either changed their email addresses or submitted erroneous ones. A small number of paper copies are issued to the legal deposit libraries and also a few members by special request. The latter is kept to a minimum as paper copies are expensive to print and post.

In the 2012-13 operating year, two issues of *The Malacologist* were circulated, plus four updates. The latter are only circulated electronically. The August issue (59) included three research grant reports and one annual award report, all in the form of short papers. There were three book reviews, one obituary (Dr Anthony Smith), one book flier and eighteen conference notices. It has become customary to report on the AGM and conference in the August issue, so this issue included abstracts of fifteen oral presentations and six posters resented at the Portsmouth conference, as well as the President's Annual Report to Council. The February issue (60) traditionally reports on the Malacological Forum. As well as a book review, two travel grant reports and one research grant report, there were fifteen pages of well-illustrated abstracts covering eighteen oral and fourteen poster presentations from this unique and fascinating meeting.

The *Updates* give interim notices of meetings, grants, awards, positions and publications. This can be quite dry, so I have started including a Malacological image in the *Update*. If members have an interesting malacological image they would like to share (it could be a Gary Lawson cartoon!), I would be pleased to receive them or any other items of malacological interest.

From the statistics presented here, it can be seen that *The Malacologist* presents a useful snapshot of the malacological research activity taking place in this part of the world, especially amongst young researchers. *The Malacologist* has ISSN number (ISSN 1759-1406) and therefore counts as a journal, so it is in the interest of awardees to present reports on their Society-funded research or travels.

2 Journal of Molluscan Studies (reported by David Reid)

The ISI impact factor for the *Journal* in 2011 rose to 1.227 (compared with 0.969 in 2010, 1.074 in 2009, 1.408 in 2008, 1.032 in 2007 and 0.968 in 2006). This is above the average for the previous 5 years (1.090) and the IF is predicted to hold up this year. The *Journal* stands at number 61 in the ISI list of 146 zoological journals (up from 73 in the previous year). These trends are in the right direction but, at relatively low values of the IF statistic, random fluctuations are to be expected. Circulation for the *Journal* in 012 was 95 institutional (of which 32 were online-only and 37 print-only) and 150 membership subscriptions (compare 107 and 138 respectively for 2011; 127 and 132 for 2010). In addition a further 2,473 institutions have electronic access to the *Journal* through publishers' consortia (includes migrated figures - compare 2,413 in 2011, 2304 in 2010), and 1,141 (compare 1,044 in 2011, 950 in 2010) have access through OUP's Developing Countries Offer (for details see http://www.oxfordjournals.org/access_purchase/developing_countries.html). This means that the *Journal* is now available to 3,859 members and institutional subscribers (compare 3,702 in 2011, 3513 in 2010).

The new pricing structure has been fixed for 2013. The cost for a combined print plus online institutional subscription is £436 (\$873); online-only subscriptions are £360 and print-only subscriptions are £399. Volume 78 (2012) contained 51 papers and research notes, totalling 380 pages (a decrease on the 400–420 pages of recent volumes). The delay between acceptance of a manuscript and electronic publication was 8 weeks. A total of 152 manuscripts was submitted in 2012 (compared with 154 in the previous year) and the acceptance rate was approximately 34%. The image of shoaling squid on the cover of Volume 78 was kindly donated by Jon Schwartz.

Members are reminded that they can access the entire electronic archive of *Journal of Molluscan Studies* (and its precursor *Proceedings of the Malacological Society of London*). Full instructions describing how to access this archive were published in *The Malacologist* in August 2007.

Our board of Associate Editors is now: Thierry Backeljau (molecular phylogenetics and genetics), Liz Boulding (population and reproductive biology), Robert Cameron (ecology and genetics of terrestrial gastropods), Richard Cook (agricultural malacology, physiology, feeding behaviour), Simon Cragg (life histories, sense organs), John Davenport (marine ecology and physiology), Mark Davies (marine ecology and behaviour), Villie Flari (physiology and behaviour), Dan Graf (freshwater bivalves), Liz Harper (marine bivalves), Bernhard Hausdorf (terrestrial gastropods), Robert Hershler (freshwater gastropods), Kurt Jordaens (systematics, ecology and pest control of terrestrial gastropods), Yasunori Kano (systematics of vetigastropods, tropical ecology), Fred Naggs (systematics and conservation of terrestrial gastropods), Manuel Malaquias (opisthobranchs), Pablo Martín (freshwater ecology, life history), Ellinor Michel (ecology, freshwater gastropods), Peter Mordan (terrestrial gastropods), Jeff Nekola (community ecology of terrestrial gastropods), Ellen Strong (freshwater and marine caenogastropods), John Taylor (Neogastropoda, mineralogy, ultrastructure), Mikael Thollesson (opisthobranchs), Janet Voight (cephalopods), Janice Voltzow (microscopic anatomy), Tony Walker (biochemistry, immunology, cytology), Suzanne Williams (molecular phylogenetics and genetics) and Nerida Wilson (opisthobranchs, deep-sea and Antarctic molluscs). I regret to report the resignation of Diarmaid Ó Foighil from the board, citing pressure of other commitments, and I thank him very much for his service. I am extremely grateful for the hard work and support of all these colleagues.

<u>The Society's website – Malacsoc.org.uk</u> (reported by Tom White)

The new Society web pages created in 2011 continue to provide important information about The Society and its activities, as well as the electronic archive of the *The Malacologist*. The only significant change to report is the addition of new pages dedicated to the forthcoming Congress of the European Malacological Societies, which will be hosted by the Malacological Society of London in September 2014 (below).

The charges levied by landl, our current Internet Service Provider (ISP) remain competitive; any small savings in hosting charges that might be made by changing provider would be outweighed by the amount of time and effort required to transfer the site. There have been no hitches with the general functioning of the website or with online transactions via our PayPal account over the last year.

Since the last AGM (21st April 2012) there have been 110,016 total visits, somewhat lower than the total for last year (130,500) which was in turn slightly lower than 2010. The number of visits is on average ~10,000 per month and this has remained relatively constant compared to previous years. A recent surge of activity has pushed the March 2013 value to 11,710 visits. The average number of pages accessed by visitors has risen slightly to 2.42 (from 1.71 last year), with an average time per visit of 46 seconds. Most visitors clearly continue to find what they are looking for relatively quickly and do not browse the site for long. As with previous years, the most popular pages have been the Home Page, the information page for the Molluscan Forum and various issues of *The Malacologist*. The Awards pages have appeared in the list of frequently accessed pages for the first time, probably due to the online application process preferred by the current Hon. Awards Secretary; this increased traffic will no doubt help publicise our Travel and Research Awards.

Data provided by the ISP document the origins of visitors to the site and how they use the pages. In 2012 direct traffic constituted 73% of visitors. These are visits from people who have either entered the site name directly into their browser or have it archived, perhaps as a 'favourite'. A further 17% arrived via links on other web pages, with the remaining 10% coming from search engines. Visitors mostly arrive from .com or .net web pages (73%) and thus their geographic location is unknown. Traffic from addresses identifiable to countries was predominantly from the UK, Ukraine, Germany, Japan, Canada and the Netherlands. Most visitors continue to land directly on a specific page (particularly issues of *The Malacologist*), reflecting the ability of search engines to locate precise information within the site.

Search terms by means of which the website was found most frequently include 'snails' (954 instances), the component words of 'Malacological Society of London' (in combination, 1449 instances) and, more interestingly, 'Praziquantel' an antihelmintic effective against flatworms (795 instances).

The web pages can be updated on request by contacting Tom White.

Euromal 2014 Website - Euromalacol/malacsoc.org.uk

New webpages dedicated to the 7th Congress of the European Malacological Societies, which will be hosted by the Malacological Society of London in September 2014, have now been constructed and will be updated over the coming weeks as details of the meeting are finalized. Construction of these pages as a sub-domain of the main website has been undertaken using Wordpress; this system is considerably more user-friendly than that used for the main webpages, and it is hoped that the whole site can eventually be migrated to the Wordpress system.

Awards (reported by Suzanne Williams)

Overall, the Society is very pleased with the number of applications that it receives for Travel Awards and Research Grants. The schemes seem to be achieving their global aim to enable young scientists to engage in malacological research activity both in the laboratory/field and at meetings. Reports from researchers funded through both schemes appear in *The Malacologist*.

The Society aims to make the following awards annually.

Travel Awards - at least 5 each of up to £500 for Society members, £300 for non-members

Research Grants - at least 5 each of up to £1500

Application forms and guidance notes for both schemes can be downloaded from The Society's website.

Travel Awards

Since the AGM in 2012 The Society has received 14 applications for awards to travel to meetings, or to undertake field or laboratory research away from the applicant's home country. The Society was able to fund the majority of these requests. All Travel Award applications are reviewed by an Awards Committee. The Society is pleased to have announced the following 13 awards.

To attend Molluscs 2012, Melbourne, 3-6 December 2012 Kennedy Wolfe £300 University of Sydney, Australia

To attend Cephalopod International Advisory Council Symposium 2012, 27th October – 2nd November 2012, Florianópolis, Santa Catarina, Brazil

Isobel Bloor £500 University of Plymouth, UK

To attend 47th European Marine Biology Symposium Arendal, Norway, 3-7th September 2012 Clarissa Fraser £500 University of Sydney, Australia

To attend the World Congress of Malacology, Azores, 21-28 July 2013

Fernando Aneiros Gonzalez £300 Campus Universitario Lagoas-Marcosende, Spain

Karen Kienberger Enayati £300 Universidad de Cádiz, Spain

Leila Carmona Barnosi £500 Universidad de Cádiz, Spain

Lauren Sumner Rooney £500 Queen's University Belfast Marine Laboratory, Ireland

Mark Anthony Phuong £500 University of California, Los Angeles, USA

Nicholas Carey £500 Queen's University Belfast Marine Laboratory, Ireland

Timothy Whitton £500 Bangor University, UK

Trond Roger Oskars £500 Bergen, Norway

Vinicius Padula Anderson £300 Zoologische Staatssammlung Munchen, Munich, Germany

To attend the Postgenomic phylogenetics EMBO Practical Course, 10-17 March 2013, Erice, Trapani, Italy Papetti Chiara £241.80 Padova University, Italy

A total of £5,441.80 was allocated by The Society for Travel Awards. All applicants have been notified of the outcome. Note that this amount does not necessarily reflect actual 'spend' as occasionally students withdraw from the intended visit.

Research Grants

By the closing date of 15th December 2012 the Society had received 24 applications from workers in 11 different countries. In general, the scientific quality of the research projects submitted was excellent. On behalf of the Society, I would like to formally thank the members of the Grants Review Panel for their hard work in reviewing all applications. The following awards (in no particular order) have been agreed by the Panel.

Martin Smith (£1250) University of Cambridge, UK

'A redescription of Burgess Shale Hyoliths and their implications for early Conchiferan evolution'

Lauren Sumner Rooney (£1500) Queen's University Marine Laboratory, Northern Ireland

'The transparent tusk shell: a tomographic model of Scaphopod anatomy

Mark Phuong (£1330) University of California Los Angeles, USA

'Does diet drive diversification in cone snails?'

Leila Carmona Barnosi (£1500) Universidad de Cádiz, Spain

'What is Aeolidia papillosa (Lineaus, 1791)?'

Suzanne Jennions (£1500) University of Bristol, UK

'Investigating the effect of ocean acidification on Mytilus californianus biomineralisation'

Kentaro Inoue (£1499) Miami University, USA

'Utilization of environmental DNA to detect endangered mussel populations in the southwest US'

Tereza Korinkova (£1477) Senckenberg Museum für Naturkunde Görlitz, Germany

'The use of molecular cytogenetics to investigate potential hybridization of slug species'

James Burgon (£1494) Natural History Museum, London

'Species diversity of *Paramelania* from Lake Tanganyika, East Africa – unifying molecular, conchological, radular and distribution data'

Therefore eight Research Grants have been funded at a total cost of £11,550. The success rate was 33%. The Grants Review Panel would like to emphasise that the quality of all applications was high and that it funded as many excellent projects as possible. Applicants will be formally notified of the outcome of their application with the next three weeks.

The Annual Award

The Society received two nominations for the 2013 Annual Award for the best PhD thesis. The unanimous decision of the Judging Panel was that the Annual Award should be given to Dr Terence P. T. Ng (University of Hong Kong) for a thesis entitled 'Reproductive Traits and Sexual Selection in the Mangrove Littorinid snails *Littoraria ardouiniana* and *L. melanostoma*''. The Society sends its best wishes and congratulations to Dr Tung, a worthy recipient.

Officers and Council

This is my first AGM as President and it has been an absolute pleasure to work with all Society Officers and Councillors during my first year in Office. All Officers and Councillors have worked extremely hard towards the continued success of The Society. This is no small task; the Society is responsible for two excellent publications, maintains a healthy membership, has an active website, provides significant numbers of grants and awards, organizes stimulating scientific malacological meetings and has also managed to maintain good financial health. I therefore sincerely thank all Officers and Councillors for their continued efforts, which is all the more notable given that each voluntarily gives their time and talents in support of The Society's objectives.

There has been some shifting of responsibility during 2012, as follows:

At the last AGM Suzanne Williams became Awards Officer, freeing up time for me to serve as President of the Society. In her first year in this role, Suzanne has done an impeccable job of managing the Society's grants and awards and I am grateful to her for taking on this role.

Over the coming year Society Officers and Council will play a key role in supporting David Aldridge with organizing the Congress of European Malacological Societies that will take place at St Catherine's College, University of Cambridge (7th – 11th September 2014). I look forward to working with all those involved in this exciting endeavour.

President	Anthony Walker
Vice Presidents	Fred Naggs
	Simon Cragg
Councillors	Mark Davies
	David Aldridge
	Robert Cameron
	Jon Ablett
	John Grahame
Hon. Secretary	Tom White
Hon. Treasurer	Katrin Linse
Membership Secretary	Richard Cook
Editor Journal of Molluscan Studies	David Reid
Editor The Malacologist	Georges Dussart
Awards Officer	Suzanne Williams
Web manager	Tom White
Archivist	Bill Bailey
Co-opt 1	Liz Platts
Co-opt 2	Richard Preece



FORTHCOMING MEETINGS

HTTP://WWW.MALACSOC.ORG.UK

Molluscan Forum

Thursday 28th November 2013 9:30 am – 6.30 pm Flett Lecture Theatre Natural History Museum, London

CALL FOR REGISTRATIONS AND PAPERS

This informal, annual, and successful meeting is designed to bring together people starting their research on molluscs, to give them the opportunity to present and discuss their work and to compare notes on methods and problems. Again the Forum will be held the day before the Young Systematists' Forum (www.systass.org/ysf), also at the Natural History Museum. This has been arranged so both meetings can be attended, although if attending both you will have to register for both meetings separately.

Attendance to the Molluscan Forum is open to all, but speakers and poster presenters should be **research students**, **post-doctoral researchers**, **undergraduate students** starting molluscan projects, and **amateurs** engaged in substantial projects that have not yet been published. Any topic related to molluscs is acceptable: palaeontological, physiological, behavioural, ecological, systematic, morphological cellular or molecular.

Short talks (~15 min) or posters may be offered. They need not be polished accounts of completed work; descriptions of new methods, work in progress, appeals for assistance with unsolved problems are equally acceptable.

In addition to talks and posters there may be opportunities to acquire books and other items contributed by members of the Society. Lunch will be provided and The Forum will end with a wine reception, both sponsored by The Malacological Society of London.

THERE IS **NO** REGISTRATION FEE AND A LIMITED AMOUNT OF HELP WITH TRAVEL COSTS WILL BE AVAILABLE FOR PRESENTERS WHO CANNOT CLAIM THEM FROM ELSEWHERE.

Enquiries and registrations to:

Prof. Tony Walker, School of Life Sciences, Kingston University, Kingston upon Thames, Surrey, KT1 2EE, UK. Tel: UK +44 (0) 208 417 2466; e-mail: t.walker@kingston.ac.uk

Non-presenters: please let us know you will be coming so that we may estimate numbers.

For more information see: http://www.malacsoc.org.uk/MolluscanForum.htm

Molluscan Forum, Thursday 28th November 2013 9:30 am – 6.30 pm Flett Lecture Theatre, Natural History Museum, London REGISTRATION FORM

Return before 4th October 2013, preferably by email to:

Prof. Tony Walker, School of Life Sciences, Kingston University, Kingston upon Thames, Surrey, KT1 2EE, UK. Tel: UK +44 (0) 208 417 2466; e-mail: t.walker@kingston.ac.uk				
Name				
Address				
Tel. No				
Email				
Status: Research Student / Undergraduate / Post-doctoral researcher / amateur (delete as appropriate)				
'Other' (please state)				
Will you attend the Young Systematists' Forum on 29 th November 2013?				
I wish to give a paper / poster (delete as appropriate) entitled:				
Please attach, as a Microsoft Word attachment, an abstract - see below for instructions - OF NOT MORE THAN 350 WORDS. Abstracts of accepted contributions will be published in the Society's Bulletin, <i>The Malacologist</i> , and on its website.				
It is expected that all oral presentations will be made using Powerpoint. If you wish to make any other form of oral presentation you MUST contact Tony Walker in advance.				
Posters should be roll-ups or mounted on stiff cards, and should need no more than a 1 metre x metre display area. They will be mounted on boards (velcro supplied).				
If you are unable to get financial support from elsewhere (students and amateurs only) and need assistance with travel costs, please enter here the cost of the cheapest possible public transport return fare to London.				
£				

Funding is not guaranteed and will be subject to a separate brief application process. Late registrations may miss the opportunity for financial support. The support will be limited, so funding from elsewhere should be sought first. A provisional programme and confirmation of registration will be sent out late October.

Abstract submission

Abstracts submitted for the Molluscan Forum should be sent as Microsoft Word files. Please use the following format:

Title (12pt, centred)

<black line>

Authors (10 pt, centred, presenting author underlined; use superscript numbers to indicate institutional affiliation)

<black line>

Institutions (10pt, centred; in this order: Number (superscript), Department, Institution, City, Country)

Presenting Author email

<black line>

Abstract (11pt, no indentation, justified, 350 words maximum)

EXAMPLE ABSTRACT

The Geographic Scale of Speciation in *Stramonita* (Neogastropoda: Muricidae)

Martine Claremont^{1,2}, Suzanne T. Williams¹, Timothy G. Barraclough², and David G. Reid¹

¹Department of Zoology, Natural History Museum, London, UK
²Department of Biology, Imperial College London, Berkshire, UK
Email: m.claremont@nhm.ac.uk

Stramonita is a relatively small, well-defined genus of muricid marine gastropods limited to the tropical Eastern Pacific and the Atlantic. The type species, S. haemastoma, is known to have teleplanic larvae and is estimated to remain in the water column for several weeks. Stramonita haemastoma shows regional variation, and this has led to the recognition of five geographical subspecies: S. h. haemastoma, from the Mediterranean and Eastern Atlantic to Brazil, S. h. floridiana, on the east coast of Florida and in the Eastern Caribbean, S. h. caniculata on the west coast of Florida and the Gulf of Mexico, S. h. rustica in the Western Caribbean and S. h. biserialis in the Eastern Pacific. The protoconch has been shown to be similar across the S. haemastoma complex, implying that all subspecies have equally long lived larvae. Within these subspecies, cryptic variation is suspected. For example, S. h. biserialis is suggested to be differentiated North/South on a small scale. In the presence of teleplanic larvae, speciation on such a small scale seems paradoxical. Various explanations for this paradox are possible. Actual (or realized) dispersal of Stramonita species may be more limited than presently believed, leading to allopatric differentiation. Alternatively, morphological differentiation may not be a reliable indicator of genetic differentiation, and S. haemastoma (sensu lato) might indeed prove to be a single taxa. It is also possible that ecological speciation could result in geographical speciation on a small scale in the presence of wide dispersal. My results suggest that five species of Stramonita are present in the Caribbean, at least three of which occur sympatrically. Gene flow is maintained between Caribbean and Mediterranean populations in at least one species, while no genetic differentiation was found along the Eastern Pacific coast. The implications of these results are discussed.



FORTHCOMING MEETINGS

Sexual Selection in Snails

Preliminary notice of the 2014 Annual General Meeting of the Malacological Society of London.

Flett Theatre, Natural History Museum, London on the afternoon of Thursday 6 March 2014.

The theme of the talks associated with the AGM will be *Sexual Selection in Snails*, and it is now confirmed that we have two leading international experts in this field as our speakers:

Prof Kerstin Johannesson, University of Gothenburg: See: http://www.bioenv.gu.se/english/staff/kejo

Dr Joris Koene, University of Amsterdam: See: http://www.falw.vu.nl/nl/onderzoek/ecological-sciences/animal-ecology/staff/joris-koene.asp



PROGRAMME

13.30: AGM

14.00: David Reid (NHM): Introduction to sexual selection

14.15-15.15: Kerstin Johannesson (Univ. Gothenburg): *Sexual selection and speciation in marine snails (Littorina species)*.

15.15-15.45: Tea

15.45-16.45: Joris Koene (Univ. Amsterdam): *Sexual selection in hermaphrodite snails*

16.45-17.00: Discussion 17.00-18.00: Wine reception

Malacological Society of London
7th Congress of the European
Malacological Societies –
EuroMal 2014
Cambridge, UK
7-11 September, 2014



The Malacological Society of London is delighted to invite you to the historic city of Cambridge for EuroMal 2014.

While talks from all aspects of malacology will be considered for inclusion in the programme, specific themes will include:

- Molluscan physiology and toxicology
- •Invasive molluscs and pests
- •Biogeography, ecology and conservation
- •Taxonomy and phylogenetics
- •Molluscs, climate and archaeology

The event will be held in the heart of the city at St. Catharine's College, with ready access to restaurants, pubs, museums and many other tourist attractions. London is just a 1hr train journey away. The College, founded in 1473, provides a wonderful mix of the old and the new, including a newly built conference centre and bar. Accommodation (single rooms only) will be available within the College, although numerous other options are available within the city.

Please note: for logistical reasons the conference will be strictly limited to 150 participants. The Congress website will soon be activated at: http://euromalacol.malacsoc.org.uk/
Details of the location can be found at: www.caths.cam.ac.uk#

Chairman:

Dr David Aldridge (St Catharine's College and Department of Zoology, University of Cambridge)

GRANTS AND AWARDS

Malacological Society of London Awards and Grants

The Malacological Society of London makes a number of Awards and Grants. These are in addition to financial support for meetings, including travel bursaries to the Molluscan Forum.

Research Grants

The Research Grants Scheme was established to commemorate the Society's Centenary in 1993. Under this scheme, the Society anticipates making at least five awards each year, each with a value of up to £1500 to support research on molluses that is likely to lead to publication. The closing date for applications each year is 15th December. Grants are preferentially conferred on students and researchers without professional positions, without regard to nationality or membership of the Society. Preference is also given to discrete research projects that fall within the subject areas covered by the Society's *Journal of Molluscan Studies*. Applications will be assessed by scientific merit, value of the project, and the extent to which the research will benefit the applicant's scientific aspirations. The successful applicants will be notified by 31st March and announced at the Annual General Meeting. The conditions of the award, notes of guidance and an application form are on the Society's website at www.Malacsoc.org.uk

Travel Awards

Travel awards are usually available as bursaries to support attendance at a conference or workshop relevant to malacology. Awards are preferentially conferred on students and researchers without professional positions. The value of each award is **up to £500**, and the Society anticipates that **at least five awards** will be made annually. The application should have the support of the project supervisor. In 2014, all travel awards will be for travel to the EUROMAL meeting in Cambridge (see page 31). For these awards, there will be one closing date on 15 May 2014. For travel awards other than to EUROMAL starting between 1st March and 31st August of the following year, the deadline is 15th December 2013. The conditions of the grant, notes of guidance and an application form are on the Society's website at **www.Malacsoc.org.uk** Preference will be given to members of the Society.

Sir Charles Maurice Yonge Awards

Successful applications for Research Grants or Travel Awards that are concerned with the study of Bivalvia may be awarded as Sir Charles Maurice Yonge Awards.

Annual Award

This Award is made each year for an exceptionally promising **initial contribution** to the study of molluscs. This is often a thesis or collection of publications. The value of the Award is £500. Candidates need not be a member of the Society but must be nominated by a member. There is no application form: the nominating member should send the material for evaluation with a covering letter or letter of support to the Honorary Awards Secretary. The closing date each year is 1st November. The winner(s) will be notified by 31st March, and announced at the Annual General Meeting.

Applications

Applications for Research Awards and Travel Grants should be sent by post, not email, to the **Honorary Awards Secretary**, Dr Suzanne Williams, Natural History Museum, Cromwell Rd., London. SW7 5BD

MALCOLOGICAL SOCIETY OF LONDON SOCIETY NOTICES

Objects

The objects of the Society are to advance education and research for the public benefit by the study of molluscs from both pure and applied aspects. We welcome as members all who are interested in the scientific study of molluscs. There are Ordinary Members, Student Members and Honorary Members. Members are entitled to receive a copy of the *Journal* and such circulars as may be issued during their membership. The society's Web Site is at:

http://www.Malacsoc.org.uk

Publications

The Society has a continuous record of publishing important scientific papers on molluses in the *Proceedings*, which evolved with Volume 42 (1976) into the *Journal of Molluscan Studies*. The *Journal* is published in annual volumes consisting of four parts which are received by fully paid-up members and student members. Members also receive The *Malacologist*, the Bulletin of the Society, twice a year.

Meetings

In addition to the traditional researches on taxonomy and systematics, new experimental, chemical and molecular techniques are amongst he topics considered for discussion meetings and papers for publication in future volumes of the *Journal*.

Subscriptions

The Annual Subscription is due on 1st January each year.

- Ordinary Members £45 (or US\$ equivalent)
- Student Members £25 (or US\$ equivalent)

Methods of Payment

- (1) Sterling cheque to "The Malacological Society of London".
- (2) Banker's standing order to: The Northern Bank (Sort code 95-01-49), 49-51 University Road, Belfast BT7 1ND, for the credit of "The Malacological Society of London" (a/c 70030422).
- (3) Credit card: Overseas members ONLY may pay by credit card: the Society can accept VISA and MasterCard payments only. Please provide the Membership Secretary with your card number and expiry date, card type (VISA or MasterCard.), the name on the card, and the cardholder's address (if this differs from your institutional address). Receipts will only be sent if specifically requested.
- Overseas members wishing to pay electronically should contact the Membership Secretary (R.COOK@KINGSTON.AC.UK) for SWIFT/BIC and IBAN numbers of our bank.

Institutional Subscriptions to the Journal

Enquiries should be addressed directly to Oxford University Press, Walton Street, Oxford OX2 6DP, U.K.

Change of Member's Address

Please inform the Membership Secretary of any change of postal or email address

Alternatively, use the address slip on the *Journal* wrapper to inform us, through Oxford University Press, of a change of address,.

APPLICATION FOR MEMBERSHIP OF THE MALACOLOGICAL SO I wish to apply for Ordinary*/Student* Membership (*delete one) I enclose a cheque payable to "The Malacological Society of London" for my first annual statement of the control of	
Title Name	
Department Institution	
Street City	
Post /Zip Code Country	
Malacological Interests	
Signature Date Please send the completed form and cheque to the Membership Secretary: Dr Richard Cook, School of Life Sciences, Kingston University, Penrhyn Road, Kingston-upon	



GENERAL NOTICES

Malacological Society of London—Awards reminder

The Malacological Society of London makes a number of Awards and Grants. Preference is given to students or those in a non-tenured position. Membership of the society is not a requirement, although Travel Awards are more generous for members.

Travel Awards are available as bursaries to support attendance at a conference or workshop relevant to malacology. Awards are preferentially conferred on students and researchers without professional positions. The maximum amount for one of these awards is £500 for Society members and £300 for non-members; the Society anticipates that at least five awards will be made annually. The application should have the support of the project supervisor. In 2014, all travel awards will be for travel to the EUROMAL meeting in Cambridge (see page 31). For these awards, there will be one closing date on 15 May 2014. For travel awards other than to EUROMAL starting between 1st March and 31st August of the following year, the deadline is 15th December 2013.

Further information—http://www.malacsoc.org.uk

Please apply via email. Dr Suzanne Williams Zoology Dept Natural History Museum Cromwell Rd London SW7 5BD United Kingdom

UK Licencing of research on cephalopods

Simon Cragg of the University of Portsmouth notes that in relation to ethics and animal experimentation, post-hatching cephalopods are now covered by Home Office regulations. The *Animals (Scientific Procedures) Act 1986* incorporates the changes made by the *Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 (SI 2012/3039)* made on 18 December 2012. A definitive version of the Act will appear in due course on http://www.legislation.gov.uk.

Job advertisement

The Delaware Museum of Natural History, of Wilmington, Delaware seeks an experienced professional to serve as Collections Manager in the Mollusc Department available in September 2013. Responsibilities include but are not limited to: maintenance and conservation of the dry and alcohol preserved mollusks and other non-vertebrate collections; responding to research inquiries regarding the collection; documentation of outgoing and ingoing loans; overseeing the care of new specimen donations; assisting with the training, supervision, and evaluation of departmental volunteers and interns; data and records management. Qualified candidates are invited to email their letter of application, CV or resume and three professional references to treed@delmnh.org or submit them to Delaware Museum of Natural History, ATTN: Human Resources, P.O. Box 3937, Wilmington, Delaware 19807. Salary requirements must be included. Acceptance of applications will begin immediately and continue until the position is filled.

USA mollusc conservation legislation

The 2012 year-end report summarizes all legislative actions taken in 2012 by the United States Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) on conservation of marine, freshwater, and terrestrial molluscs as they are applied by the U.S. Endangered Species Act (ESA) and other relevant legislation. Digital copies of each installment are available directly on the American Malacological Society conservation web page http://www.malacological.org/conservation.html via the AMS Conservation Committee Imperilled Species Newsletter link. AMS membership is not required to view each post.

