Assessment of the doctoral dissertation

by Emily Yi-Shyuan Chen, M.Sc.

„Life at both edges of the globe – Bipolarity concept tested on pelagic ostracods"

Despite significant progress in research on diverse biota of the marine pelagic zone, our understanding of the origins of current biodiversity patterns and biogeographic distribution of many marine zooplankton taxa remains limited and unevenly developed across regions. Both the Arctic and Antarctic are examples of such underexplored regions. These polar areas, though different in many respects, are currently experiencing rapid environmental transformations driven by global climate change. One potentially overlooked consequence of this change is the disruption of a biogeographical pattern involving the disjunct distribution of the same or closely related taxa in both polar regions, a phenomenon known as bipolarity. Advancing knowledge on bipolarity in marine pelagic ecosystems requires multifaceted research efforts, including large-scale sampling campaigns, meticulous taxonomic identification of morphologically challenging taxa, advanced morphological analyses, numerical assessments of biota-environment relationships, and labor-intensive genetic and bioinformatic studies. While clearly valuable, such investigations are costly, time-consuming, and demand a high level of dedication, making the engagement of researchers willing to undertake this essential work particularly important. In this context, the research undertaken by Emily Yi-Shyuan Chen, the International Master of Science in Marine Biological Resources 2020, is both timely and well-justified, particularly as it focuses on the pelagic marine family Halocyprididae, a relatively diverse yet understudied group of myodocopid ostracod microcrustaceans, which are widely distributed in both polar regions and thus well-suited for exploring the bipolarity concept.

Ms Emily Chen's doctoral dissertation (hereafter referred to as 'PhD Thesis') "*„Life at both edges of the globe – Bipolarity concept tested on pelagic ostracods"*, submitted for evaluation, was prepared under the supervision of Prof. Dr hab. Katarzyna Błachowiak-Samołyk and Dr hab. Emilia Trudnowska at the Institute of Oceanology of the Polish Academy of Sciences.

The structure of the PhD Thesis is logical and coherent. It begins with a comprehensive Summary in English (and in Polish), which includes an introduction, clearly stated research aims, a synopsis of the conducted studies, a summary of main findings, and a list the scientific publications. This is followed by four core chapters presented as multi-authored scientific publications (each with three to five authors), accompanied by supplementary materials. The PhD Thesis concludes with the declarations of authors' contributions to each co-authored paper and an unnecessarily duplicated list of research papers. The overall presentation is clear, well-organized, and easy to navigate. The narrative flows logically and cohesively, and, with the exception of a minor issue involving puzzling page numbering, the PhD Thesis is accessible and well-structured. The Summary section introduces the research topic, presents a sound justification for the work, and clearly outlines both the general and specific research objectives. It also accurately reflects and separately summarizes the content of each research paper. However, the PhD Thesis lacks a dedicated synthesis chapter, that would integrate the findings from all four studies, contextualize them within the framework of bipolarity, and evaluate the extent to which the research objectives have been met. In my view, the final paragraph of the synopsis does not adequately fulfill

this integrative function, and the inclusion of a separate synthesis chapter would have significantly strengthen the thesis and substantiated the conclusions more convincingly.

The main body of the PhD Thesis consists of four research papers, three of which have already been published (2023-2025) in *Journal of Biogeography*, *Journal of Plankton Research* and in *Marine Biology*. Notably, the first paper was published in a Q1 journal in the field of *Ecology*, while the two subsequent papers appeared in Q2 journals in the category of *Marine and Freshwater Biology*, according to the *Journal Citation Reports*. The fourth paper is currently in manuscript form and has been recently submitted to the journal of *Molecular Ecology*. The PhD candidate is the first and lead author on all four papers and played a major role in each study, from conceptualization and data analysis to manuscript writing and revision. These contributions are well-documented and sufficient to evaluate the PhD candidate's individual input within the context of the thesis. I would, however, be interested to know whether the PhD candidate was personally responsible for identifying the species, sex, and developmental stage of the specimens used in the research, or whether the study relied on pre-identified material and existing databases.

In her PhD thesis, Emily Chen promises to contribute meaningful insights into the concept of bipolarity using a hypothesis-driven approach, i.e. testing, as she wrote on p. 6, "*whether polar pelagic ostracods have underlying biological and genetic divergences shaped by unique environmental pressures at each pole; if so, these divergences challenge the assumption of ecological and evolutionary symmetry across polar regions and suggest that oceanographic changes may differentially disrupt reproductive stability and genomic integrity in these cold-adapted taxa*". While the Summary includes a clear and concise statement of the research problem and outlines a set of specific objectives, it primarily serves to underscore the importance of the conducted work and its relevance to advancing knowledge on bipolarity. The detailed research questions, however, emerge more clearly within the individual research papers, each of which includes comprehensive literature reviews that frame the specific hypotheses and research gaps addressed in the study.

Research paper no. 1 presents an extensive literature-based review of bipolar and antitropical distribution patterns among marine taxa, ranging from bacteria to mammals. Using a rigorous search strategy with well-defined relevance and eligibility criteria, the authors identified 221 relevant publications from an initial pool of 2,506 items published between 1800 and 2024. These records were retrieved from three large public databases and two Arctic and Antarctic marine species registers. The 221 selected papers included 148 records classified as representing bipolar distribution pattern, mostly at the species level (54%), while the remaining 517 records described antitropical distributions. Among 123 recent (non-palaeontological) bipolar records, the most frequently reported taxa were cnidarian Hydrozoa (20%), Foraminifera (19%), and arthropods (17%, with copepods constituting 11% of the total). However, the taxonomy used throughout the main text and supplementary materials was not fully consistent, limiting comparability between sections. For example, Fig. 4 lists Rhizaria among the most frequent bipolar taxa, yet this name does not appear in the supplementary Table S1, creating confusion. Another minor issue is the inconsistent taxonomic attribution of certain taxa, which may hinder reader comprehension. For instance, being an ostracodologists, I was unfortunately unaware that *Doridunculus* belongs to the nudibranch gastropods, and *Rhabdoon reesi* to cnidarians. Given the context of the PhD Thesis which promises to test the bipolarity concept using ostracods as a model group, ostracods are barely mentioned in this review, having no published bipolar records. Also, in consideration of the terminological confusion surrounding bipolarity (and antitropicality), clearly

documented by the authors, and the stated aim to clarify an standardize the terms, one may feel that the „concept“ of bipolarity was rather poorly defined as „an area“ in the dedicated section 2.1 Definitions: “*For the purpose of this study, bipolarity was defined as the area* (my emphasis) *above the Arctic Circle (66°33'N) and below the Antarctic Circle (66°33'S) (Figure 1).*”. Despite these minor issues, the paper is a well-executed synthesis, offering carefully verified data on the disjunct distributions of marine organisms. It provides a solid foundation for further research on the bipolarity (and antitropicality) phenomenon, especially relevant in light of climate change, which may severely alter these distribution patterns. This review demonstrates the PhD candidate’s familiarity with the field, her ability to synthesize complex data, and a sound application of the literature to her own research.

Research paper no. 2 investigates sex ratios in four Arctic and four Antarctic ostracod species of the family Halocyprididae. The study is based on an exceptionally large dataset comprising nearly 18,500 specimens, both adults and pre-adults (A-1, the final juvenile stage), collected from approximately 500 zooplankton samples obtained during seven polar expeditions spanning from 1983 to the present. Based on this global dataset, without considering sample level variation, the authors report that all eight species exhibited female-biased sex ratios (ranging from 61% to 90% females), significantly deviating from an expected 1:1 ratio. The authors also tested the fit of a 2:1 female-to-male model, commonly observed in freshwater ostracods. This model was rejected for three species, two of which had significantly higher females proportion and one with a lower proportion than expected under the 2:1 model. However, one additional species, *Boroecia maxima*, in Table II also shows a significant deviation from the 2:1 based on the reported p-value, although the mean and variation do not strongly support such deviation. I would be interested to hear the PhD candidate clarify this point during the defense.

To further contextualize the observed female bias, the study examines the global sex ratio variation across multiple axes: spatial variation within the Arctic (based on two datasets), temporal variation (based on a time series in the Arctic Kongsfjorden, Svalbard), depth-related differences (Arctic vs. Antarctic), seasonal variation (Arctic vs. Antarctic), and developmental stage (A-1 juvenile vs. adults). The overall findings consistently indicate an average numerical dominance of females (60-70%) across species, depth zone, and season, although some seasonal variability was noted. These results are novel and significant, suggesting that consistent overall female-biased sex ratios in polar pelagic marine halocyprid ostracods may result from similar underlying mechanisms in both polar regions, as the authors concluded. Nevertheless, due to the scarcity of similar studies on non-polar myodocopid ostracods, it remains possible that such ratio biases are widespread across this group, not unique to polar regions. Despite this general female dominance, substantial variability was also observed. For example, *Boroecia* spp. showed extreme variation in sex ratio across Arctic stations (from 0% to 78% female), based on composite data from samples collected at different depths, seasons and years. Marked seasonal trends based on composite data from all species of a given polar region was observed as well, with the highest adult female proportion occurring in summer and the lowest in spring, though statistical tests for these differences were not provided. The authors also report notable differences in the sex ratios between A-1 juveniles and adults. These differences were evaluated both in aggregate (across four Arctic species in the Kongsfjorden time series) and at the individual species level based on the total counts (from several samples), yet again without statistical testing. Given the scale and diversity of the dataset, it is surprising that primary statistical analyses were not employed to explore

the homogeneity of sex ratios across individual samples prior to combining counts (juveniles and adults) from different samples, depths, seasons or collecting sites. For instance, testing whether A-1 and adult sex ratios were consistent across different samples at the same station, or whether seasonal sex ratios of individual species were homogeneous across different years, would have provided a more nuanced understanding. As the most abundant samples inevitably have a greater influence on global averages, failure to test these assumptions limits the interpretative strength of the results. The dataset certainly supports more complex statistical modeling, which could help disentangle or partition the various environmental and biological factors potentially influencing sex ratios. Including raw counts of females and males per individual sample in supplementary material (as was done in Research paper no. 3) would allow readers to independently assess sample-level variability. The currently provided supplementary Table S1 falls short in this regard, and illustrates how rough some of the comparisons are: e.g., 355 individuals of all species from all autumn samples of one Arctic dataset are compared to 5,661 individuals of all species from all summer samples from three Arctic datasets covering different years, yet no weighting or error estimation is applied. Furthermore, there are some inconsistencies between the supplementary Table S1 and figures. For example, Table S1 and Fig. 2 report 1,835 specimens for the Antarctic winter, whereas Fig. 7 appears to show this number for the summer. Similarly, the Antarctic depth range 500-1000 m is shown as having 7,020 specimens in Table S1, but 7,002 in Fig. 2. For *Boroecia maxima*, the total number of specimens is listed as 3878 in Table S1 and 3877 in Fig. 2. While the figures are well-designed and employ a range of visualization techniques (box plots, pie charts, density plots, bar charts), greater consistency in how a single variable (i.e., female proportion) is represented would facilitate interpretation across different dimensions (season, depth, time series, developmental stages).

Despite these issues, I have no doubt that the authors have convincingly demonstrated a general female-biased sex ratio in these polar ostracods. During the defense, I would like to hear the PhD candidate's interpretation of the causes behind this bias. What hypotheses might explain higher female proportion in A-1 juveniles compared to adults, and what could account for seasonal variation in female proportion. For example, the lowest female proportion in the Antarctic winter was attributed to "*post-reproductive mortality of males*", but this would imply a relative increase in females rather than their decrease. I look forward to the PhD candidate's clarification on this point. Finally, regarding the statistical methods, I would appreciate the PhD candidate could clarify the application of Pearson correlation, which is mentioned in the Material and Methods section but not clearly presented in the Results. Please also address this during the defense.

Research paper no. 3 is the first study to thoroughly and meticulously document frequent sexual abnormalities (i.e. occurrences of "pseudopenises") in females at the final juvenile stage (A-1) in four polar pelagic ostracod species of the family Halocyprididae from summer samples collected between 2010 to 2019 at various depths from two stations in the Arctic Fram Strait. The morphological analyses were carried out with great care and precision. The correct identification of the sex of A-1 individuals, including those with atypical sexual characteristics related to the occurrence of pseudopenis, is beyond doubt. The typical and atypical morphologies of the A-1 females and males were clearly and convincingly illustrated with high-quality figures. The incidence of sexual abnormalities was remarkably high, ranging from 12.3% to 16.6% of the combined number of adult and A-1 individuals per species. The highest proportion was observed in *Boroecia maxima*, with a statistically significant difference compared to the other species. The authors attributed the lowest proportion of abnormalities

in *B. borealis* to small sample sizes. However, I find this explanation unconvincing, particularly in case of *O. obtusata*, which had an even smaller sample size but a higher frequency of abnormalities. In comparing the frequency of abnormalities across depth strata, it would have been preferable to analyze this relationship for each species separately, rather than pooling all species collected from a given depth. Nonetheless, the observed frequency of sexual abnormalities is striking, not only due to the lack of prior reports of such phenomena in ostracods, but also because of broader biological implications. This pattern raises important questions. Are these abnormalities indicative of developmental disruptions that could affect fertility and/or survival? Or are they a typical aspect of the developmental biology of this group, possibly with some adaptive significance? Since these abnormalities were detected exclusively in the A-1 individuals, the proportion of last instars bearing pseudopenis ranged between 34% and as much as 40% in the studied species. This proportion would be even higher when calculated relative only to A-1 females. From this perspective, it is noteworthy that no such abnormalities were observed in 300 individuals of three (?) species of the same family from historical Antarctic samples collected in 1930s and 1970s. However, the authors did not specify how many A-1 females were included in these Antarctic samples, making it difficult to assess whether the absence of abnormalities reflects limited small sample size, regional differences, or other factors, such as historical timing of collection or exposure to anthropogenic stressors.

I would welcome a more detailed explanation from the PhD candidate during her defense regarding potential environmental drivers, including climate change and pollution, that might lead to increased intersexuality or developmental instability in marine microcrustaceans. I would also appreciate clarification of the final paragraph on p. 5 of this paper, which is somewhat unclear: "*In recent years, there has been an increase in first records of intersexuality in gonochoristic taxa such as shrimp (Paschoal & Zara 2017) and porcelain crabs (Ferreira & Guzmán 2013), which were attributed to genetic anomalies due to low numbers of intersex specimens.*". Furthermore, as a non-specialist on appendage morphology of Myodocopa, I would ask the PhD candidate to elaborate on the sexual dimorphism of the first antenna (antennule) in this group, as the current description on p. 3-4 lacks sufficient details for non-experts: "*The frontal organ and attached first antenna are also important features because for females, it consists of eight short antennules and two long antennules of the same length while males have six long and irregular length antennules*.".

Research paper no. 4 presents novel sequencing of complete mitochondrial genomes for five pelagic halocyprid ostracod species from Arctic and Antarctic regions. The study revealed several notable features, including species-specific variation in the tRNA secondary structures, a strong A+T bias, and a distinctive gene arrangement, including the presence of an intron in the *nd3* gene, which appears to be unique for halocyprids. Given that complete ostracod mitochondrial genomes are known only for seven species, four of which belong to the subclass Myodocopa, this study provides crucial new data for understanding genetic diversity and phylogenetic relationships within Myodocopa, the class of Ostracoda, the superclass Oligostraca, and more broadly, the Pancrustacea. In my opinion the methodologies employed were appropriate, clearly aligned with the research objectives, and consistent with current genomic standards. The combination of laboratory work and advanced bioinformatics analyses required a high degree of technical skills, which the PhD candidate demonstrated competently, particularly through effective use of various software packages, including R. The results are scientifically robust, the interpretations are sound and nuanced, and the conclusions are well-supported by appropriate sample sizes and statistical analyses. I have no serious concerns regarding

this work, aside from just a few minor editorial comments: some diversity indices are not described in the Material and Methods section (e.g., Tajima's D, Fu's F or haplotype diversity Hd and average number of nucleotide differences K), and a few typographic issues remain (e.g., unnecessarily italicized commas in line 283; "represent\tative" in line 362, a missing period in line 482, "haploytype" in line 559, and slightly awkward phrasing "concentrated region in the South China Sea" in lines 556-557). These are all easily correctable. I hope that this manuscript will be accepted for publication in the near future.

I was particularly intrigued by the finding of substantial intraspecific variation in mitochondrial DNA, and even more so by the gene arrangements in the studied halocyprid species, which were markedly more divergent from the ancestral model, exhibiting numerous positional changes and inversions, than those observed in the podocopid freshwater families. I would be interested to hear whether the PhD candidate has any hypotheses to explain these striking differences among ostracod lineages, and such high intraspecific diversity and haplotype richness in the studied halocyprids, despite the apparent absence of cryptic species in the studied samples.

Overall assessment and final recommendation

In summary, this PhD thesis constitutes a conceptually innovative and methodologically rigorous investigation into the reproductive biology, morphological variation, and genomic architecture of marine pelagic ostracods of the family Halocyprididae. I fully concur with the PhD candidate conclusion, stated on page 6 in the last sentence of the summary "these studies advance the foundational understanding of halocyprid ostracods at both poles and highlight their importance in marine biodiversity and evolutionary research". While the individual components of the Thesis are scientifically robust and make significant contributions to our understanding of bipolarity and polar ostracod biology, it should be also noted that the overarching hypothesis to rigorously test the concept of bipolarity was addressed only to a limited extent. As a result, there is a slight mismatch between the Thesis title and the outcomes of the research.

The Emily Yi-Shyuan Chen's doctoral dissertation offers an original solution to a significant scientific problem and demonstrates that the PhD candidate possesses capacity for independent scientific research work, as well as a deep and comprehensive understanding of relevant theoretical framework. Each of the four main chapters is either already published (three) or submitted to the reputable peer-reviewed journal (one), attesting to the scientific quality and maturity of the work. Based on my expertise in aquatic invertebrate evolutionary ecology, I confidently recommend this dissertation to the competent authorities of the Scientific Council of the Institute of Oceanology of the Polish Academy of Sciences for defense and support the subsequent steps required to confer the doctoral degree.

Prof. Dr. Habil. Tadeusz Namiotko

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