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EXPLORING SPECIES BOUNDARIES IN THE DIATOM GENUS *RHOICOSPHENIA* USING MORPHOLOGY, PHYLOGENY, ECOLOGY, AND BIOGEOGRAPHY

by

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A thesis submitted to the

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This thesis entitled:
Exploring species boundaries in the diatom genus *Rhoicosphenia*
using morphology, phylogeny, ecology, and biogeography
written by Evan William Thomas
has been approved for the Department of Ecology and Evolutionary Biology

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Dr. Andrew P. Martin

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Dr. Steven K. Schmidt

Date_____________

The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Abstract

Thomas, Evan William (Ph.D., Ecology and Evolutionary Biology)

Exploring species boundaries in the diatom genus *Rhoicosphenia* using morphology, phylogeny, ecology, and biogeography

Thesis directed by Professor Andrew P. Martin

Certain taxonomic groups within the American flora and fauna are relatively unexplored in terms of their biodiversity – one of these groups are the diatoms. The diversity, phylogeny, and ecology of the common freshwater diatom genus *Rhoicosphenia* are explored. While determining the diversity of *Rhoicosphenia* in American streams, several new taxa have been discovered, both from fossil and recent collections. These discoveries are discussed in the context of the history of the genus, its diversity in other parts of the world, and the morphological characters used to identify species distinctions. Prior to this dissertation, one taxon (*R. abbreviata*) was commonly reported from the United States, and after these studies, eight morphologically distinct taxa were found. Presently, no published molecular studies have sequenced any species of *Rhoicosphenia* for use in phylogenetic analyses. In the literature, four historical hypotheses (dating back to the erection of the genus in 1860) about its position in the diatom tree of life remain untested by molecular data. This dissertation used morphological and multi-marker molecular data to test the four hypotheses and place *Rhoicosphenia* in the phylogeny of diatoms. The results did not fully support any of the four hypotheses, but did offer insight into parts of the diatom tree that have been underexplored. *R. abbreviata* was reported from nearly all floristic treatments of diatoms of the US, but little quantitative data was provided in regards to its niche. Due to its presence in a large percentage of studies, it has often been referred to as a geographically cosmopolitan species with broad tolerance of ecological parameters. Large water quality monitoring datasets were analyzed to understand the biogeographical patterns of the eight new taxa as well as their ecological niches. Results suggest that none of the taxa are cosmopolitan and none are broadly tolerant of all ecological conditions, but there is variation in both range size and ecological tolerance among the eight taxa. Traditionally, diatom species have been described based largely on morphological variation of their silica cell walls, but the results of the many aspects of this dissertation provide evidence for a more robust, unified species concept for diatoms that relies on many different types of data in addition to morphology, including geographical distributions, ecological preferences, and phylogenetics.

Dedication – This dissertation is dedicated to my children: Alexander, Julian, and Everett. You can do anything you want in life, as long as you ensure that you are kind to all you meet.

Acknowledgments – During the time working on my dissertation I have encountered many people who are exceedingly deserving of thanks, which I will attempt to provide in this section. My wife, Jennifer Ress, who has been by my side before I started my dissertation and is by my side today – your encouragement and assistance in all aspects of our lives helped make this dissertation possible – I love you. My boys, Alexander, Julian, and Everett, you are all so special to me! No matter how long or short of a day I spent working on my dissertation or teaching, you were *always* ready to play when Daddy came home. Let's play some more now that this is all done! To my parents, siblings, grandparents, and friends – you always let me tell you about my dissertation and were usually slightly interested, thank you. Dr. Andy Martin – you allowed me to use my creativity to pursue my interests and I am very grateful for the time we worked together. To my committee members, Dr. Steve Schmidt, Dr. Sam Flaxman, Dr. Nancy Emery, and Dr. Steve Lamos, I greatly appreciate your time and advice. To my many research mentors, past and present, thank you for imparting upon me the skills to observe my surroundings, ask questions, and plan projects to study the unstudied. To Dr. Patrick Kociolek, who provided many opportunities for me to pursue my passion and guided me through many years of my PhD. I am grateful for the Jessup Award allowing me access to *Rhoicosphenia* across the US while at the Diatom Herbarium of the Academy of Natural Sciences in Philadelphia, with special thanks to Dr. Marina Potapova for her support and encouragement. To the EBIO department at CU, which is full of amazing people – faculty, graduate and undergraduate students, and staff, many of whom directly or indirectly helped me realize my goal of finishing my dissertation – thank you all so much. Finally, to all those not mentioned directly, thank you!

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CHAPTER I

INTRODUCTION

In the United States, there are approximately 158 genera of freshwater diatoms and over 2000 species reported from water quality monitoring datasets (ANS et al. 2011–2016). These taxa are comprised of both benthic and planktonic forms, are found in lotic and lentic habitats of varying quality from oligotrophic to eutrophic (Round & Sims 1981, Round et al. 1990). If we are to consider all diatom diversity from the US, including fossil taxa and taxa found in soil, wet rock faces, and even on animals, the 2000 names in the ANS list would be low estimate of diversity. As a group, diatoms span gradients of low conductivity freshwater to high salinity inland and marine environments, acid and alkaline habitats, and cold to hot temperatures (Round et al. 1990). Some taxa are broadly distributed across the landscape and tolerant of broad ecological conditions, others are known from very few locations, while others live in very specific habitats. The diatom communities living in one very small area of one habitat could be from the same genus or be from any part of the diatom tree of life, meaning that the genetic diversity of the diatom community within any one location is great. It is clear that the diversity of diatoms in the US is vast, but what remains constant is that the diatoms present in any location are suited to that ecological space. When living, these diatoms carry out their lifecycle as primary producers and serve as the base of many aquatic, and thus terrestrial, ecosystems. The morphological diversity of diatoms is also vast, and traditionally three major lineages were recognized, the “centric,” “araphid,” and raphid diatoms. Molecular phylogenetic studies have shown that morphological descriptors (e.g., “centric” and “araphid”) are not accurate representations of the diatom phylogeny as they represent non-monophyletic lineages (Sims et al. 2006, Theriot et al. 2009, Theriot et al. 2015). Within all lineages of diatoms there are a variety of forms. For example, within raphid diatoms, some species have raphes on both valves (biraphid) while others have a raphe on one valve (monoraphid). Raphe arrangement can also vary, with some raphes set in either a canal or keel. Of all of the possible diatom genera and species to examine, this dissertation offers detailed insight into the morphological, molecular, and ecological diversity of the raphid genus *Rhoicosphenia* Grunow (1860). This dissertation seeks to address four major question and does so with a series of journal articles that are published (Chapters 2–4) or in preparation (Chapter 5).

Chapter 2 is a review of the history of the genus and the present state of *Rhoicosphenia* taxonomy and diversity. The major question posed by the introduction can be summarized as “what is the diversity of *Rhoicosphenia* in freshwaters of the United States?” *Rhoicosphenia* was described over 150 years ago (Grunow 1860), and in the time since, approximately 50 named taxa have been described (Guiry 2016). While approximately 87% of diatom genera are restricted to either freshwater or marine habitats (Round & Sims 1981), *Rhoicosphenia* differs from many other genera in that it lives in both freshwater (Levkov et al. 2010, Thomas & Kociolek 2015) and marine (Ligowski et al. 2014, Thomas & Ligowski 2016) habitats. Since 1981, there has been an increase in described genera, and, therefore, it is likely that a higher proportion of genera are known from either freshwater or marine habitats but not both (Snoeijs & Weckström 2010). While this chapter is focused on the diversity of freshwater *Rhoicosphenia*, research has been done determining how species within genera that span the salinity gradient are related and how many times they have changed from freshwater to marine, or vice-versa, over their evolutionary history (Alverson et al. 2007). We also do not know whether the ancestor of *Rhoicosphenia* was a freshwater or marine diatom, however many closely related genera have been found to be freshwater (Thomas et al. 2016). While this dissertation cannot cover all aspects of *Rhoicosphenia*, the new species discoveries presented are worth noting and should have an effect on how the diversity within the genus is viewed moving forward. Ultimately, the second chapter highlights two published papers that described five species from the western United States and also presents descriptions and discussions of five more undescribed species that have not yet been published.

The third chapter seeks to address the phylogenetic position of *Rhoicosphenia* in the diatom tree of life. Four historical hypotheses for the position of *Rhoicosphenia* have been posited over the years, the first when the genus was erected in 1860 (Grunow 1860), and a few more before 1900. These four hypotheses were summarized in the early 1980's, when several papers on *Rhoicosphenia* biology were published (Mann 1982a, Mann 1982b, Mann 1984). Only two attempts have been made to test those hypotheses using morphological data, one with only five taxa and eleven characters (Kociolek & Stoermer 1986), the other with 49 taxa and 35 characters (Cox & Williams 2006). The analysis presented in this dissertation uses both morphological and multi-marker molecular datasets. The results indicated that *Rhoicosphenia* is most closely related to 'monoraphid' diatoms, including members of the genus that, in the late 1800's, was referred to as *Achnanthes* (now *Achnanthidium*), and that *Rhoicosphenia* is basal to the clade of diatoms that includes *Gomphonema*, the Cymbellales. These results were supported with topology testing. We also tested the hypothesis that 'monoraphid' diatoms are monophyletic, which was rejected, with *Achnanthes sensu stricto* being more closely related to members of the Bacillariales and distantly related to other 'monoraphid' diatoms. It is also interesting that early diatomists did not fully realize the relationship between 'monoraphid' diatoms and the Cymbellales. However there was one diatomist, Mereschkowsky (1902) that used chloroplast morphology and demonstrated this relationship, which the results of our study support.

The fourth chapter addresses the phylogeny of the diatom family Rhoicospheniaceae Chen & Zhu (1983) where the genus *Rhoicosphenia* is placed. Even though there is a rich history of diatom phylogenetics with both morphological and molecular data, the monophyly of the Rhoicospheniaceae has not been addressed. Despite the lack of phylogenetic analyses to address this question, ten genera in addition to *Rhoicosphenia* have been added to the family, including *Campylopyxis* Medlin, *Chelonicola* Majewska, De Stefano & Van de Vijver, *Cuneolus* Giffen, *Epiphalaina* Holmes, Nagasawa & Takano, *Gomphonemopsis* Medlin, *Gomphoseptatum* Medlin, *Gomphosphenia* Lange-Bertalot, *Poulinea* Majewska, De Stefano & Van de Vijver, *Rhoiconeis* Grunow, and *Tursiocola* Holmes, Nagasawa and Takano (itis.gov, Guiry 2016). This chapter (Majewska et al. 2015) was originally submitted without me as an author, but after review, I was asked to join the authors on the manuscript in order to test the phylogenetic position of two new genera, *Chelonicola* and *Poulinea*, as part of the Rhoicospheniaceae. The subsequent phylogenetic analysis for which I gathered and analyzed data, and wrote relevant results and discussion provided the editor and reviewers with compelling data to publish the paper. The analysis of the family Rhoicospheniaceae that I performed included morphological observations on the genera *Cuneolus*, *Gomphonemopsis*, *Gomphoseptatum*, and *Rhoicosphenia*, which were included in Round et al. (1990), as well as *Gomphosphenia* and other non-related taxa to determine the monophyly of the Rhoicospheniaceae as currently circumscribed. The results of the cladistic analysis based on morphological characters suggested that the Rhoicospheniaceae is non-monophyletic. The implications of this on the diatom classification scheme are also discussed in the chapter.

The fifth and final chapter studies the ecological and biogeographical patterns of *Rhoicosphenia* in the United States. Due to the perception of low species diversity in the US, only *R. abbreviata/curvata* has been reported in most studies (Patrick & Reimer 1966, Lowe 1974, Lawson & Rushforth 1975, Benson & Rushforth 1975, Czarnecki & Blinn 1977, Clark & Rushforth 1977, Kaczmarska & Rushforth 1983, Reavie & Smol 1998, ANS et al. 2011–2016). Lowe (1974) wrote an often cited work on the ecological preferences of diatoms and the guide was meant to be used to inform water quality analyses based on the compilation of detailed niche requirements. However, in the “Recommendations” preceding the ecological data on diatoms of the US, he cautioned that the data he presented is not static and will change over time (Lowe 1974). This chapter may be the impetus for that change in regards to *Rhoicosphenia* diversity in the US, as with the description of more taxa, and their inclusion in future monitoring projects, the data on *R. abbreviata* in Lowe (1974) will likely no longer serve the monitoring community well. The null hypothesis for this chapter was that *R. abbreviata/curvata* has a broad ecological niche and biogeographic range. My hypothesis was that there are many species of *Rhoicosphenia* in the US with varying niche requirements. For this analysis, two large sets of ecological data and *Rhoicosphenia* distributions from the state of California and from the remainder of the US, were used to determine the niche requirements of three described species of *Rhoicosphenia* along with five undescribed morphotypes discussed in the second chapter. These eight taxa along with water quality data were graphically displayed using an ordination technique, Non-metric Multidimensional Scaling (NMDS), which was followed by an Analysis of Similarity (ANOSIM) on the 28 taxa pairwise comparisons. The resulting NMDS plot and ANOSIM output rejected the null hypothesis that the eight species all had overlapping niches. In fact, less than half (12) of the pairwise species comparisons had similar niches, while the other 16 pairs had statistically different niches. The results of this chapter are two-fold. First, the additional five new *Rhoicosphenia* morphologies coupled with the three extant species described from California, statistically reject the null hypothesis that there is only one broadly distributed and ecologically tolerant species of *Rhoicosphenia* in the US. Second, many of these species have a multivariate niche that is distinct from other *Rhoicosphenia* species. These results, along with detailed distributional data may allow for the acquisition of more accurate species identifications in water quality assessments that better reflect the conditions of the studied waterbodies. However, for this to happen, those in pursuit of the most accurate water quality monitoring results must recognize the increased specific diversity that is documented by diatom taxonomists.

Summary remarks

Prior to this dissertation, the diatom genus *Rhoicosphenia* was well known from many (thousands) locations in the US (ANS et al. 2011–2016), but only as the species *R. abbreviata/curvata*. Also, the systematics of *Rhoicosphenia* had only been assessed with morphology, despite widespread use of molecular data for diatoms. Further, the Linnaean classification of the Rhoicospheniaceae was untested and genera were being placed in it based on little more than hunches. Finally, no attempt at fine-scaled taxonomy (all *Rhoicosphenia* were *R. abbreviata/curvata*) coupled with ecological data was made to address the niche of any species. In this sense, every *Rhoicosphenia* was everywhere in the US, and it didn’t really matter if the environment selected them or not because of the “broad tolerance” of the only species ever reported. Through this dissertation, it is my intention to encourage others to closely examine species, especially the common ones that we may think are well understood. In completing these analyses of other species and genera, we will likely gain more knowledge, and much more quickly than we have gained over the past several generations of diatom studies in the US.

CHAPTER II

HISTORY AND TAXONOMY OF *RHOICOSPHENIA*

Introduction and taxonomic history

The diatom genus *Rhoicosphenia* Grunow (1860) was erected based on a previously described species, *Gomphonema curvatum* Kützing, as the generitype. *Gomphonema* Ehrenberg (1832) and *Rhoicosphenia* share a morphological similarity in that they are both (often) asymmetrical to the transapical axis of their valve face, meaning they look wedge-shaped. Grunow distinguished this new genus, *Rhoicosphenia*, based on his observations that in girdle view (side-view) *Rhoicosphenia* is "saddle-shaped", or bent, and also noted that the central nodule in *Rhoicosphenia* is only present on the concave valve face, not on both valves as is the case with *Gomphonema* species (Grunow 1860, pg. 511). These two distinguishing characters have had a profound influence on phylogenetic hypotheses involving both genera up to the present day (Grunow 1860, Mann 1982a, Schütt 1896, Van Heurck 1896). In the time since the erection of *Rhoicosphenia* as a distinct genus and prior to the start of this dissertation, 29 names are currently accepted taxonomically out of a total of 45 names in the AlgaeBase.org database (Guiry 2016). This number is relatively modest when compared to the number of species and infraspecific names of some other raphid diatom genera, e.g. *Navicula* Bory 1293–7107, *Neidium* Pfitzer 168–326, *Gomphonema* Ehrenberg 421–1423, *Nitzschia* Hassall 763–1346, *Amphora* Ehrenberg ex Kützing 357–1201, *Pinnularia* Ehrenberg 676–2707 (first number in range is number of currently accepted names, second number is total number of names in database)

(Guiry 2016). Of the currently described *Rhoicosphenia* taxa approximately 1/3 are from freshwater habitats, approximately 1/3 are from marine habitats, and some are difficult to discern the type of habitat from which they were described. In terms of the geographical distribution of descriptions, 28 described from Europe, 5 from Asia, 3 from North America, 2 from Australia, 1 from South America, 1 from Africa, and 1 from Antarctica. From 1860 to 1976, 51 taxa were described, and it wasn't until 2007–2010 that the next seven species were described (Levkov et al. 2007, Levkov & Nakov 2008, Levkov et al. 2010).

Up until 2009, prior to the beginning of this dissertation, one extant species of *Rhoicosphenia* was commonly reported from the US – *R. abbreviata* (Agardh) Lange-Bertalot (1980) (ANS et al. 2011–2016). In 1980, this species was synonymized (Lange-Bertalot 1980) with another commonly reported congener, *R. curvata* (Kützing) Grunow (1860). In the literature, there is a trend of only *R. curvata* (Foged 1966, Patrick & Reimer 1966, Lawson & Rushforth 1975, Benson & Rushforth 1975, Czarnecki & Blinn 1977, Clark & Rushforth 1977) being used in floristic treatments prior to 1980, and after 1980, some diatomists began adopting the name *R. abbreviata* (Reichardt 1984, Wenter 1990, Hofmann 1994, Cocquyt 1998, Cumming et al. 1995, Reavie & Smol 1998, Novelo et al. 2007), while others continued to use *R. curvata* (Foged 1984a, Foged 1984b, Kaczmarska & Rushforth 1983).

Despite the lack of *Rhoicosphenia* diversity shown in these publications, several *Rhoicosphenia* had been described and reported from the US in the late 19th century. *Rhoicosphenia curvata* var. *gracilis* M. Schmidt in Schmidt et al. (1899) and the freshwater fossil diatoms *Rhoicosphenia curvata* f. *minor* M. Schmidt in Schmidt et al. (1899), and *Rhoicosphenia curvata* var. *subacuta* M. Schmidt in Schmidt et al. (1899) were all described from the western US by Schmidt and *Rhoicosphenia curvata* var. *major* Cleve was described from a sample from Oregon (Cleve 1895). Of these taxa previously known from the US, only *R. curvata* var. *subacuta* has been reported outside of the type location (Stoermer & Yang 1969). In addition to freshwater members of the genus, *Rhoicosphenia genuflexa* (Kützing 1844) Medlin in Medlin & Fryxell (1984) and *Rhoicosphenia marina* (Kützing 1844) M. Schmidt in Schmidt et al. (1899) are two marine species reported from coastal marine waters of the US. Therefore, the known diversity of *Rhoicosphenia* in the US is greater than just *R. abbreviata*, however, the other taxa are not, with any regularity, identified from studied locations within the US. One potential explanation for these previously mentioned taxa not being reported in floristic surveys and ecological datasets from the US could be their synonymy, along with dozens of other *Rhoicosphenia* taxa into *R. curvata* (Van Landingham 1978).

Current understanding of *Rhoicosphenia* diversity in the US

This dissertation has led to the description of five published new *Rhoicosphenia* species from the United States, a doubling of previously known diversity. *Rhoicosphenia patrickae* E.W. Thomas & Kociolek in Thomas et al. (2015) and *Rhoicosphenia reimeri* E.W. Thomas & Kociolek in Thomas et al. (2015) were described from a fossil deposit in Oregon. Three extant *Rhoicosphenia* were described from streams in California, *Rhoicosphenia californica* E.W. Thomas & Kociolek, *Rhoicosphenia lowei* E.W. Thomas & Kociolek, and *Rhoicosphenia stoermeri* E.W. Thomas & Kociolek. The following text, and corresponding images, were originally published in two separate journal articles; the three extant taxa in: Thomas, E.W. & Kociolek, J.P. 2015. Taxonomy of three new *Rhoicosphenia* (Bacillariophyta) species from California, USA. Phytotaxa 204: 1–21, and the two fossil taxa in: Thomas, E.W., Kociolek, J.P. & Karthick, B. 2015. Four new *Rhoicosphenia* Grunow species from fossil deposits in India and North America. *Diatom Research* 30: 35–54. Pagination and figure and table numbering have been modified for presentation in this dissertation. Two fossil species from India were also described in Thomas et al. (2015); their descriptions, but not their images, have been included in this dissertation.

Taxonomy of three new *Rhoicosphenia* (Bacillariophyta) species from California, USA

Evan W. Thomas & J. Patrick Kociolek

Abstract

Nearly two centuries of diatom floristic and ecological studies in North America have resulted in the recognition of relatively few *Rhoicosphenia* species. Three new species of *Rhoicosphenia* are described from water quality monitoring samples from streams across the state of California. *Rhoicosphenia stoermeri* is large, with a panduriform central area. *Rhoicosphenia lowei* is also large, but the valve is narrower than *R. stoermeri*, and its central area is smaller. *Rhoicosphenia californica* is narrow and linear and the most commonly encountered of the newly described species. Light and scanning electron microscope observations of these new species with comparisons to previously described taxa, coupled with ecological and distribution data from across the state, highlight the overlooked *Rhoicosphenia* diversity in North America. The ultimate goal of this work is to aid in refined taxonomic identifications within the genus with the possibility of increased resolution in ecological studies using diatoms.

Keywords: *Rhoicosphenia*, *Bacillariophyta*, *diatom*, *endemic*, *streams*, *pseudocryptic*, *dichotomous key*

Introduction

Rhoicosphenia Grunow (1860) is a diatom genus that is commonly reported in freshwater ecosystems of the United States and is distributed across the country. *Rhoicosphenia* has been reported from the West (Sovereign 1958, Patrick & Reimer 1975, Leland et al. 2001, Bahls 2009), Southwest (Czarnecki & Blinn 1977, 1978, Czarnecki 1979), Mountain West (Benson & Rushforth 1975, Lawson & Rushforth 1975, Patrick & Reimer 1975, Clark & Rushforth 1977, Grimes & Rushforth 1982), Great Lakes (Wujek 1967, Stoermer et al. 1999), Northeast (Patrick & Reimer 1975, Reavie & Smol 1998, Potapova & Charles 2002), and Southeast (Hendricks et al. 2006, Johansen et al. 2007). However, out of the 27 species and approximately 30 intraspecific taxa currently described and listed in the *Catalogue of Diatom Names* (Fourtanier & Kociolek 2011), only two species, *Rhoicosphenia abbreviata* (Agardh 1831) Lange-Bertalot (1980) and its synonym *R. curvata* (Kützing 1833) Grunow (1860) account for the vast majority of records in the previously listed studies. These studies suggest that *Rhoicosphenia* diversity in the US is low and that the morphological diversity and ecological niche of the commonly reported *R. abbreviata* and *R. curvata* are broad.

In terms of other *Rhoicosphenia* taxa reported from the United States, five have been described as new; *Rhoicosphenia curvata* var. *gracilis* M. Schmidt in Schmidt et al. (1899), *Rhoicosphenia curvata* f. *minor* M. Schmidt in Schmidt et al. (1899), *Rhoicosphenia curvata* var. *subacuta* M. Schmidt in Schmidt et al. (1899), *Rhoicosphenia patrickae* E.W. Thomas & Kociolek in Thomas et al. (2015), and *Rhoicosphenia reimeri* E.W. Thomas & Kociolek in Thomas et al. (2015). Most of these taxa are known only as fossils in the US. Only *R. curvata* var. *subacuta* has been reported in an extant sample and it only made up 0.00238% relative abundance of the sample in which it was found (Stoermer & Yang 1969). The 'cosmopolitan' species *Rhoicosphenia genuflexa* (Kützing 1844) Medlin in Medlin & Fryxell (1984) and *Rhoicosphenia marina* (Kützing 1844) M. Schmidt in Schmidt et al. (1899) are two marine species reported in coastal marine waters of the US.

Globally, *Rhoicosphenia* is commonly reported in freshwater (e.g. Lawson & Rushforth 1975, Rivera 1983, Foged 1984a, Gil-Rodríguez et al. 2003, Hu & Wei 2006, Al-Handal & Wulff 2008, Harper et al. 2012), brackish (Levkov et al. 2010), and coastal marine ecosystems (Misra 1956, Giffen 1970, Medlin & Fryxell 1984a) and can be found on every continent. Prior to the description of US fossils (Thomas et al. 2015), the most recently described *Rhoicosphenia* species had been found in Europe and Asia (Levkov et al. 2007, 2010, Levkov & Nakov 2008). Similar to the reports of *Rhoicosphenia* in the US, most reports from around the world are of *R. abbreviata* or *R. curvata* and are not other previously described species (Lawson & Rushforth 1975, Rivera 1983, Foged 1984a, Gil-Rodríguez et al. 2003, Hu & Wei 2006, Al-Handal & Wulff 2008, Harper et al. 2012).

Sampling efforts in freshwater rivers in California have produced several hundred diatom samples for water quality monitoring purposes through the Surface Water Ambient Monitoring Program (SWAMP) and Southern California Bight (SCB) Project. During the enumeration of these samples, the morphological diversity of *Rhoicosphenia* specimens was great. All new species described in this paper are compared to *Rhoicosphenia abbreviata* as documented and illustrated by Levkov et al. (2010). The purpose of this paper is to present light and scanning electron microscope observations and describe the taxa encountered as new to science. Finally, a discussion of cryptic and pseudocryptic species, as well as undescribed taxa in the genus *Rhoicosphenia* is presented.

Materials and methods

Samples examined for this study come from two water quality monitoring programs in California. One study concentrated on coastal watersheds in the Southern California Bight (SCB) from Santa Barbara in the North, San Diego in the South, San Bernardino in the East, and the

Pacific Ocean in the West. The other samples are part of the Surface Water Ambient Monitoring Program (SWAMP) throughout the state of California. Both of these studies collected diatoms from natural substrates using the United States Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP; Peck et al. 2006). Therefore, the samples collected were "non-targeted", meaning that sub-samples at one locality came from a variety of in river habitat types (pools, riffles, runs, etc.), as well as a variety of substrates (sand, gravel, cobbles, boulders, plants, woody debris, coarse particulate organic material, etc.) and were collected with tools and techniques described in Peck et al. (2006). Sampling for both projects included ecological and physical habitat parameter measurements. These studies generated several hundred algal collections from streams in California between 2007 and 2012 and the 205 samples with *Rhoicosphenia* populations investigated in this study can be found in the original publication, with the same samples, but different ecological variables in Appendix A of this dissertation. This table in the original publication lists the samples observed in this study, includes information on which species are found in which samples, and includes location and ecological data as well. Specifically, the data available in this document are Project (SCB or SWAMP), Sample ID, Site Name, Sample date, Latitude, Longitude, Elevation (meters), pH, Conductivity (µS/cm), Nitrate & Nitrite (mg/L), and Orthophosphate (mg/L) (Original publication). A summary of the species and their ranges, means, and median values for Elevation, pH, Conductivity, Nitrate & Nitrite, and Orthophosphate can be found in Table 1.

Species	Elevation (m.a.s.l.)	pH	Conductivity (µS/cm)	Nitrate & Nitrite (mg/L)	Orthophosphate (mg/L)
R. stoermeri	R: 29–1226 M: 370 Md: 353	R: 7.4–8.8 M: 8.2 Md: 8.0	R: 111.9–2325.0 M: 468.9 Md: 327.5	R: 0.0052–0.0483 M: 0.0153 Md: 0.0105	R: 0.0084–0.1400 M: 0.0331 Md: 0.0255
R. lowei	R: 11–1491 M: 473 Md: 352	R: 7.3–8.8 M: 8.2 Md: 8.2	R: 78.7–1142.0 M: 346.0 Md: 221.4	R: 0.0067–0.2470 M: 0.0499 Md: 0.0268	R: 0.0212–0.1950 M: 0.0610 Md: 0.0552
R. californica	R: 6–2683 M: 539 Md: 337	R: 6.7–9.0 M: 7.9 Md: 8.0	R: 40.5–4028 M: 513.2 Md: 279.0	R: 0.0035–7.7800 M: 0.2169 Md: 0.0262	R: 0.0057–0.4480 M: 0.0617 Md: 0.0437

Table 1: Ecological data for observed distributions of new *Rhoicosphenia*. A summary of the ecological data for observed distributions of new *Rhoicosphenia* species described in this paper. Ranges (R), arithmetic means (M), and median (Md) are included for Elevation (meters), pH, Conductivity (µS/cm), Nitrate & Nitrite (mg/L), and Orthophosphate (mg/L).

In addition, a KML/KMZ file for use with Google Earth (Google, Inc.) is included as Supplemental Materials Document 1 (this electronic file is not included in the dissertation, but can be found associated with original publication) which provides an interactive map of the locations of these *Rhoicosphenia* species distributions in California.

Holotype slides and material are deposited at the Academy of Natural Sciences (ANSP), Philadelphia, Pennsylvania, and isotype slides and type material are housed in the Kociolek Collection at the University of Colorado, Museum of Natural History. A summary of the type locations and material can be found in Table 2.

Taxon	Location	Latitude (º)	Longitude (º)	Date Collected
R. stoermeri	Bear Creek	34.24154	-117.88599	Nov. 5, 2007
R. lowei	Ash Creek	41.134220	-120.80025	Sept. 15, 2010
R. californica	Big Chico Creek	39.72855	-121.88105	June 30, 2008

Table 2: Type Locations for new species from California. All locations are in California, USA.

For light (LM) and scanning electron microscopy (SEM) observations, algal collections were boiled in nitric acid to remove organic material and clean diatom frustules, settled and rinsed with deionized water until pH was neutral. For LM observations, diatoms were air dried onto cover glasses, and permanently mounted in Naphrax®. LM was performed using an Olympus® BX51 Photomicroscope (Olympus America Inc., Center Valley, Pennsylvania) with differential interference contrast optics. Specimen images were captured at 432 pixels/inch with an Olympus® DP71 Digital Camera attached to the Olympus® BX51 and a computer. SEM was performed with cleaned specimens air dried onto cover glasses, attached to aluminum stubs, sputter-coated with 5 nm of gold-palladium and examined in high vacuum mode using a JEOL JSM 6480LV low vacuum SEM (JEOL Ltd, Tokyo, Japan) with an accelerating voltage of 15 kV and a JEOL JSM 7401 field emission SEM (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 5 kV. SEM was performed at the Nanomaterials Characterization Facility, University of Colorado, Boulder. All images in this paper are from the type material. Terminology for the valves and copulae of *Rhoicosphenia* follows Ross et al. (1979), Cox & Ross (1981), Mann (1982), Levkov et al. (2010) and Thomas et al. (2015). A dichotomous key to the species described in this paper, as well as *Rhoicosphenia abbreviata*, is included in the Results section following the species descriptions.

Results

A summary of morphological traits of new taxa described and taxa they are compared to can be found in Table 3.

Taxon	Source	Habitat	Distribution	Length	Width	Striae (R)	Striae (D)
R. stoermeri	This paper	Freshwater & brackish	California	25–84	6–9	11–13, 14–16	11–12, 14–15
R. lowei	This paper	Freshwater	California	16–75	5–8	9–11	9–11
R. californica	This paper	Freshwater & brackish	California	8–50	3–6	11–12, 13–15	9–11
R. marina var. intermedia M. Schmidt	Schmidt 1899	Marine	California	51–66	9–10	13–14	14–17
R. curvata var. *subacuta* M. Schmidt	Schmidt 1899	Marine	China, Europe, North America	34–76	6.5–9	8–15	9–15
R. affinis Levkov	Levkov et al. 2010	Freshwater	China	34–65	6.5–8.5	11–14	11–14
R. lacustris Levkov	Levkov et al. 2010	Freshwater & brackish	Lake Dojran, Macedonia	25–62	6–9	13–15	13–15
R. baicalensis Skabichevskii	Levkov et al. 2010	Freshwater	Lake Baikal	16–49	3.5–5.5	9–12	10–12
R. reimeri E.W. Thomas & Kociolek	Thomas et al. 2015	Freshwater, fossil	Oregon	18–70	7–10	9–11	9–11
R. macedonica Levkov & Krstic	Levkov et al. 2007	Freshwater	Lake Ohrid, Macedonia	15–52	5.5–8.5	18–22	22–24
R. tenuis Levkov & Nakov	Levkov et al. 2010	Freshwater	Lake Ohrid, Macedonia	15–60	3–5	12–16	13–17
R. abbreviata (C. Agardh) Lange-Bertalot	Krammer & Lange-Bertalot 1986	Freshwater	Germany	10–75	3–8		
R. abbreviata	Levkov et al. 2010	Freshwater	Germany	14–52	5–7	9–12	9–12
R. abbreviata	Reavie & Smol 1998	Freshwater, low conductivity	Common St. Lawrence River	12–40	4–6	15	17

Table 3 (part 1): Taxon comparison and trait summary of California taxa. Information on habitat and morphology of the three new species of *Rhoicosphenia* as well as taxa used for comparison.

Taxon	Source	Habitat	Distribution	Length	Width	Striae (R)	Striae (D)
R. curvata (Kützing) Grunow	Benson & Rushforth 1975	Freshwater, lotic & lentic	Huntington Canyon, Utah	18–35	4–7	11–13 c, 17–20 p	
R. curvata	Boyer 1927		Widespread	15–25		15	16
R. curvata	Clark & Rushforth 1977		Widespread	15–40	4–8	10–15	10–15
R. curvata	Czarnecki & Blinn 1977	Freshwater, wide conductivity	Lower Lake Powell, Colorado River, Arizona & Utah	12–75	4–8	15	15
R. curvata	Czarnecki & Blinn 1978	Freshwater, wide conductivity	Widespread Arizona	12–75	4–8	15	15
R. curvata	Fungladda Kaczmars-ka & Rushforth 1983	Majority of samples	Widespread	15–17	3–3.5	9–12	
R. curvata	Kaczmars-ka & Rushforth 1983	Freshwater, estuarine, marine		20	4.5	15–19	
R. curvata	Lawson & Rushforth 1975	Freshwater, lotic	Provo River, Utah	20–70	4–10	8–9 c, 11–12 p	
R. curvata	Patrick & Reimer 1966	Freshwater	United States	12–75	4–8	9–15	11–13

Table 3 (part 2): Taxon comparison and trait summary of California taxa. Information on habitat and morphology of the three new species of *Rhoicosphenia* as well as taxa used for comparison.

Rhoicosphenia stoermeri **E.W. Thomas & Kociolek,** ***sp. nov.*** (Figs 1–37)

Frustules clavate and slightly flexed in girdle view. Valves heteropolar in valve view, narrowly lanceolate to lanceolate with elongated acute apices in larger specimens, smaller specimens oblanceolate with head pole more blunt and rounded than acute foot pole, 25–84 µm long, 6–9 µm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). R-valve: raphe filiform with minor undulations, proximal raphe ends 3–10 µm apart, dilated externally, crook-shaped internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area narrow at apices, becoming wider towards central area, central area elongated and panduriform, ovate in smallest specimens. Striae radiate in center of the valve and parallel at apices, 11–13 striae in 10 µm at center of valve, 14–16 striae in 10 µm at apices, composed of lineolate areolae, 30 in 10 µm. D-valve: raphe branches 4–6 µm long at head pole, not extending beyond pseudoseptum, and 5–7 µm long at foot pole, external proximal ends slightly inflated, internal proximal ends crook-shaped in same direction and distal ends not inflated externally, terminate in helictoglossae internally. Striae parallel in middle, slightly radiate at apices, 11–12 striae in 10 µm in middle of valve, 14–15 striae in 10 µm at apices, composed of lineolate areolae. Both valves with pseudosepta at each apex, 3–13 µm long. Both valves with apical pore field at foot pole, porelli 4 per 1 µm. Girdle bands open.

In the SEM, external views of the R-valve (Figs 23, 25–27) show valve outline and large panduriform central area. Proximal raphe ends on R-valve (Figs 23, 26) are dilated and drop-shaped. The axial area is narrow near the ends, becoming inflated around the proximal raphe ends, becoming narrow again in between them (Figs 23, 26). Distal raphe ends on the R-valve continue onto the mantle (Figs 25, 27). Apical pore fields are present only at the foot pole and porelli are linear and obliquely arranged (Fig. 27). Internally, the valvocopula is modified to fit over and near entirely cover the pseudoseptum and has an aperture at the head pole (Figs 24, 28, 30). Internal valve views show panduriform central area and proximal raphe ends strongly hooked in the same direction (Figs 24, 29). Also, the areolae can be seen in troughs between the virgae (Fig. 29). External views of the D-valve show the shortened raphe branches and lineolate areolae (Figs 31, 33, 34). Distal raphe end on D-valve terminates on valve face at head pole (Fig. 33) and continues onto mantle at foot pole (Fig. 34). Proximal raphe ends on D-valve (Figs 31, 33, 34) are dilated and drop-shaped. Internal SEM of the D-valve shows troughs between the virgae as well as lineolate internal openings to the external areolae (Fig. 32). Pseudosepta are present at each pole and raphe branches extend beyond the pseudosepta at each pole (Figs 35, 36). The valvocopula is modified to cover pseudosepta on the valve interior (Figs 35, 36). Interior views of head and foot pole with crook-shaped internal proximal raphe ends (Figs 35, 36). In girdle view, valve flexure is illustrated and girdle elements are each ornamented with one row of simple poroids (Fig. 37).

Type: USA. California: Bear Creek, Los Angeles County, 34.24154º N, 117.88599º W, collected by M. Brady, A.E. Fetscher, J.P. Kociolek & E.W. Thomas, November 5, 2007 (holotype ANSP! Circled specimen on slide GC 65218 made from ANSP GCM 5696, illustrated in Fig. 10; isotype JPK! 2627, slide and material, University of Colorado, Museum of Natural History, Kociolek Collection, Boulder, Colorado, USA).

Etymology: This species is named in honor of Dr. Eugene F. Stoermer, one of the true leaders in research on diatoms.

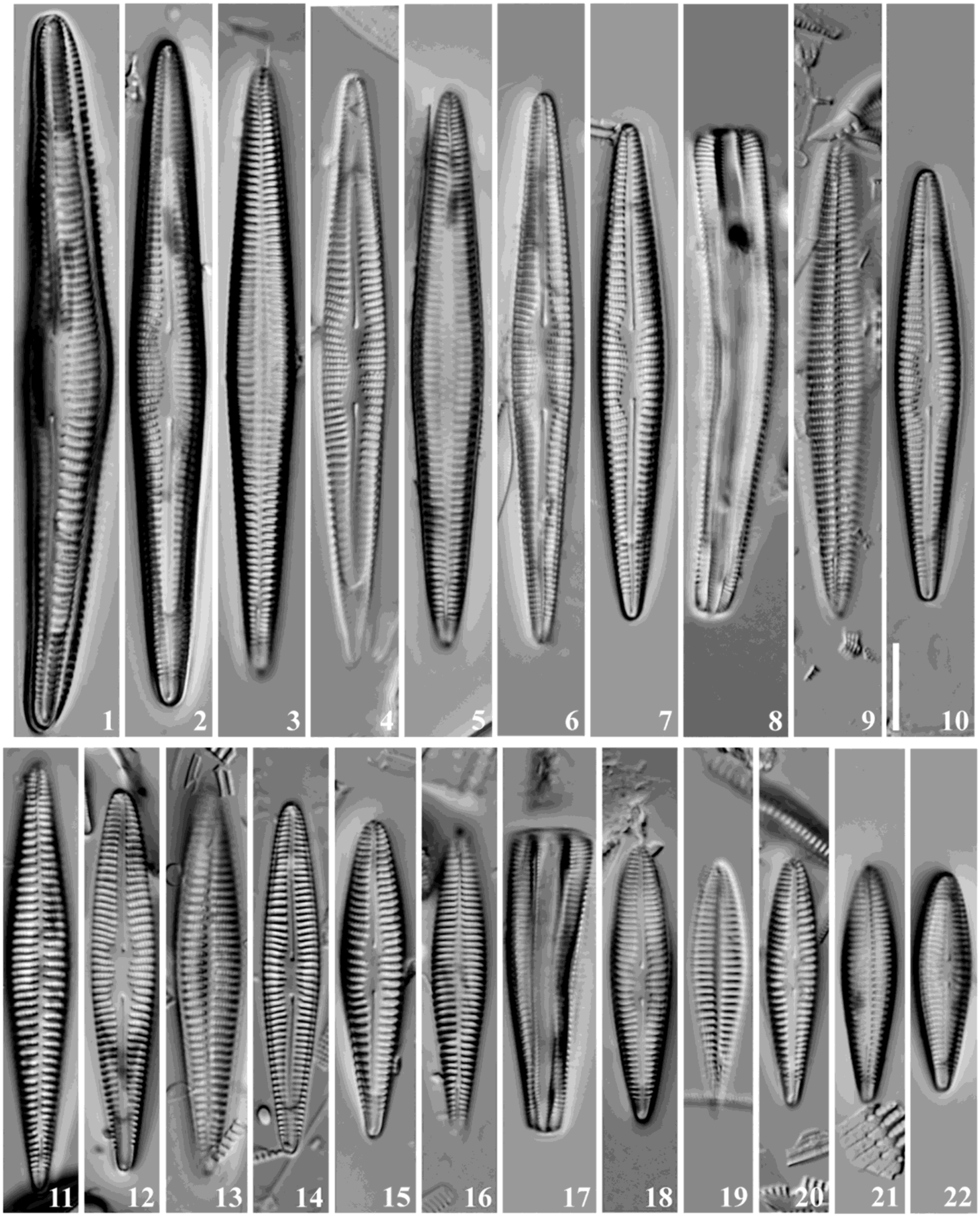
Taxonomic remarks: *Rhoicosphenia stoermeri* is distinguished from other *Rhoicosphenia* taxa by its shape, size and large panduriform central area. Morphologically, *R. stoermeri* most closely resembles *R. marina* var. *intermedia* M. Schmidt (1899, Pl. 213, Figs 36–39). However, there are 11–13 striae in 10 µm at the center of the valve in *R. stoermeri* and 14–16 striae in 10 µm in *R. marina* var. *intermedia*. In addition, in specimens of similar size (lengths of approximately 50–65 µm) the shape of *R. stoermeri* valves is lanceolate while *R. marina* var. *intermedia* valves are oblanceolate. In terms of ecology, M. Schmidt reports *R. marina* var. *intermedia* as being found in marine environments of 'coastal California' and *R. stoermeri* is found in the San Gabriel Mountains of Southern California in very low conductivity streams.

Rhoicosphenia stoermeri is also morphologically distinct from *R. curvata* var. *subacuta* M. Schmidt. The specimens of *R. curvata* var. *subacuta* that are most similar to *R. stoermeri* are from marine environments of China ('Insel Hainan', Schmidt 1899, Pl. 213, Figs 6–7). These two taxa are similar in shape, but *R. stoermeri* is distinguished by its large panduriform central area, distance between proximal raphe ends, and larger valve size. *R. stoermeri* also has more dense striae, 11–13 striae per 10 µm, as opposed to 9–11 striae per 10 µm in *R. curvata* var. *subacuta*.

Rhoicosphenia stoermeri is also similar to *R. affinis* Levkov in Levkov et al. (2010), but the shape of *R. affinis*, 'subclavate, with attenuate and subprotracted head pole' (Levkov et al. 2010) distinguishes it from *R. stoermeri*, especially with regard to the head pole. *R. stoermeri* is most similar in its morphology to *R. lacustris* Levkov (Levkov et al. 2010), but it has less dense striae 11–13 per 10 µm (as opposed to 13–15 per 10 µm in *R. stoermeri*) and has a greater size range 25–84 µm (vs. 25–62 µm in *R. stoermeri*). *Rhoicosphenia stoermeri* has a more distinctly panduriform central area with greater separation between proximal raphe ends as compared with *R. lacustris*.

Compared to the type material of *R*. *abbreviata* as documented by Levkov et al. (2010), *R*. *stoermeri* can be distinguished by several features. First, the size range of *R*. *stoermeri* is 25–84 µm long and 6–9 µm wide, both longer and wider than reported for *R*. *abbreviata* at 14–52 µm long and 5–7 µm wide. Second, the narrowly-lanceolate to lanceolate valve shape distinguishes *R*. *stoermeri* from linear to narrowly clavate valves of *R*. *abbreviata*. Striae density is also different between the two; *R*. *stoermeri* has distinctly punctate striae, 11–13 in 10 µm at the center while *R*. *abbreviata* has 9–12 in 10 µm at the center and are not distinctly punctate (Levkov et al. 2010).

Distribution and ecological notes: Found in lower elevation sites from Los Angeles to Redding, CA. Most sites are close to the Pacific Ocean with the exception of three sites and are generally characterized by low nutrients, slightly alkaline, and low conductivity.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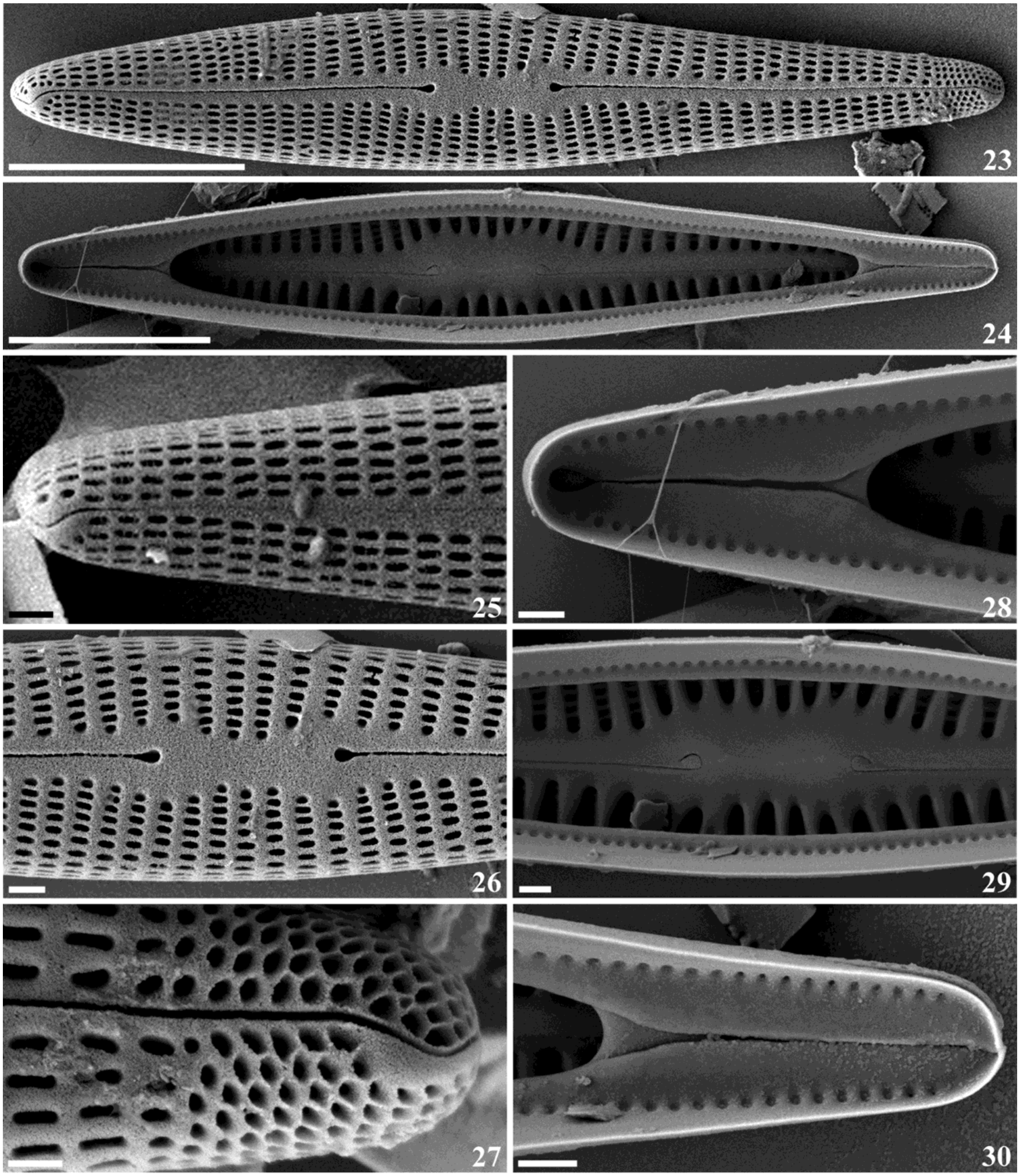
Figures 1–22: Type material of *Rhoicosphenia stoermeri* from Bear Creek, Los Angeles County, California, USA. LM. 10. Holotype specimen. 1, 2, 4, 6, 7, 10, 12, 14, 15, 18, 20, 22. R-valves. 3, 5, 9, 11, 13, 16, 19, 21. D-valves. 8, 17. Girdle views. Scale bar is 10 μm.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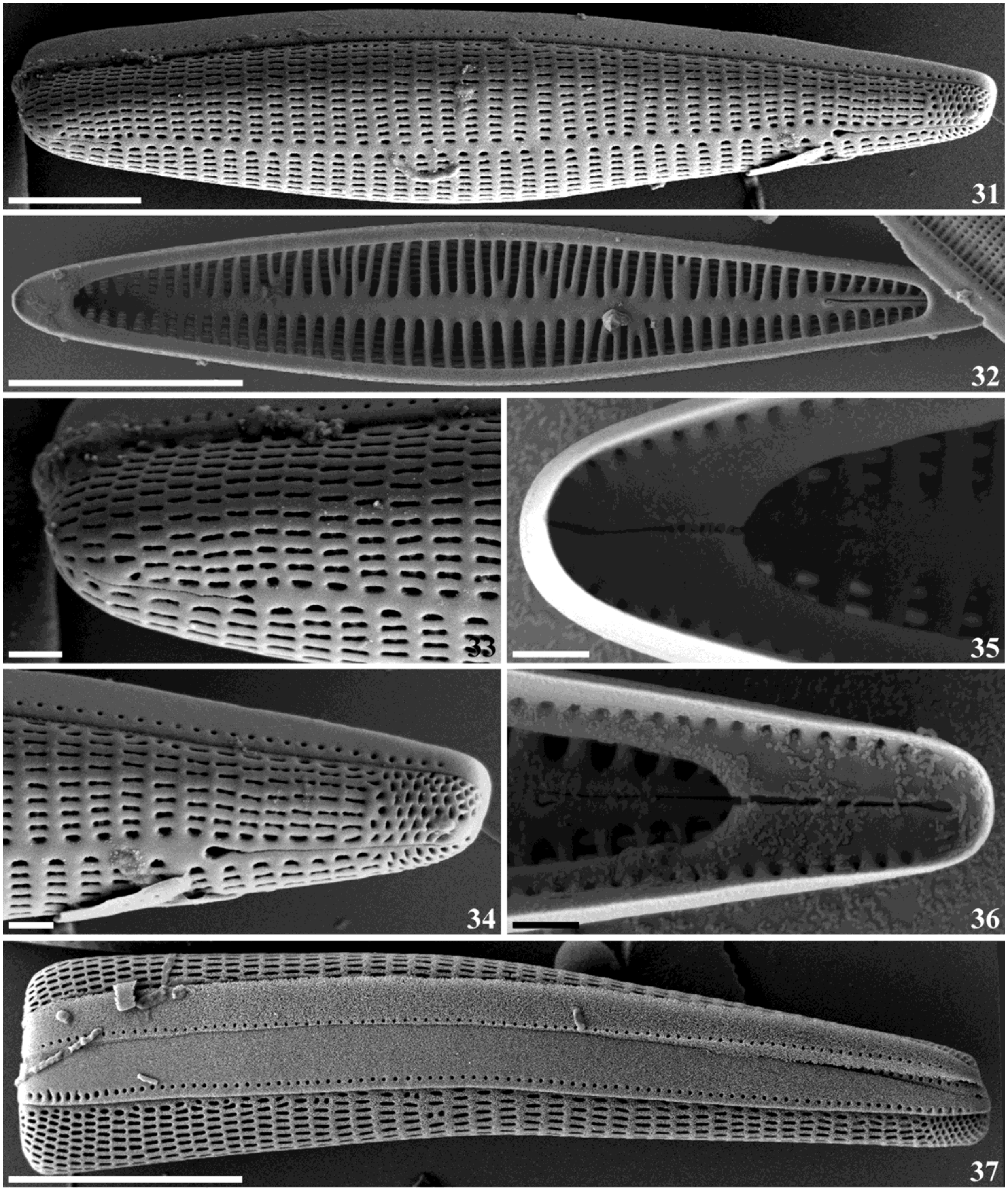
Figures 23–30: Type material of *Rhoicosphenia stoermeri* from Bear Creek, Los Angeles County, California, USA. SEM. 23, 25–27. External R-valve outline, panduriform central area, and dilated proximal raphe ends (23, 26). Distal raphe ends on the R-valve continue onto the mantle (25, 27). Apical pore fields are present only at the foot pole and porelli are linear and obliquely arranged (27). 24, 28–30. Internally, the valvocopula is modified to fit over and near entirely cover the pseudoseptum and has an aperture at the head pole (24, 28, 30). Internal valve views show panduriform central area and proximal raphe ends strongly hooked in the same direction (24, 29). The areolae can be seen in troughs between the virgae (29). Scale bars are 10 μm (23–24) and 1 μm (25–30).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Figures 31–37: Type material of *Rhoicosphenia stoermeri* from Bear Creek, Los Angeles County, California, USA. SEM. 31, 33, 34. External views of the D-valve show the shortened raphe branches and lineolate areolae (31, 33). Distal raphe end on D-valve terminates on valve face at head pole (33) and continues onto mantle at foot pole (34). Proximal raphe ends on D-valve (31, 33, 34) are dilated and drop-shaped. 32, 35, 36. Internal D-valve with troughs between the virgae as well as lineolate internal openings to the external areolae (32, 35). Pseudosepta are present at each pole and raphe branches extend beyond the pseudosepta at each pole (32). Valvocopula modified to cover pseudosepta on valve interior (35, 36). Interior views of head and foot pole with crook-shaped internal proximal raphe ends (35, 36). 37. In girdle view, valve flexure is illustrated and girdle elements are each ornamented with one row of simple poroids Scale bars are 10 μm (32, 37), 5 μm (31), and 1 μm (33–36).

Rhoicosphenia lowei* E.W. Thomas & Kociolek, *sp. nov. (Figs 38–74)

Frustules clavate and slightly flexed in girdle view. Valves heteropolar in valve view, oblanceolate to linear-clavate with bluntly rounded head pole and rounded foot pole, 16–75 µm long, 5–8 µm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). R-valve: raphe filiform, proximal raphe ends inflated, crook-shaped internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area narrow at poles widening central area, central area oval, two to three times broader than axial area, and sometimes constricted in center creating two distinct lobes. Striae radiate in center of the valve and slightly radiate throughout, 9–11 in 10 µm at center of valve and are composed of lineolate areolae, 30 in 10 µm. D-valve: raphe branches 2–3 µm long at head pole, not extending beyond pseudoseptum, and 5–7 µm long at foot pole, external proximal ends not expanded, internal proximal ends crook-shaped in same direction and distal ends not inflated externally, terminate in helictoglossae internally. Striae parallel in center, radiate at apices, 9–11 in 10 µm at center of valve, and are composed of lineolate areolae. Both valves with pseudosepta at each apex, 3–7 µm long at head pole and 2–6 µm long at foot pole. Both valves with apical pore field at foot pole, porelli 3 per 1 µm. Girdle bands open.

In the SEM, external views of R-valve (Figs 59–62) show the bluntly rounded head pole, lineolate areolae, and dilated proximal raphe ends. The apical pore field is present only at the foot pole and the porelli are more densely arranged, smaller, and rounder than the stria areolae. The distal raphe ends on R-valve continue onto the mantle (Figs 60, 62). Valvocopula is modified to overlap with the pseudosepta at each end of valve (Figs 63, 65, 66). The internal proximal raphe ends are strongly crook-shaped in the same direction (Fig. 64). The external of the D-valve is characterized by the shortened raphe branches and lineolate areolae (Fig. 67). The distal raphe end on D-valve terminates on valve face and the head pole (Fig. 68) and continues onto the mantle at the foot pole (Fig. 69). The proximal raphe ends are dilated and drop-shaped (Figs 68–69). In girdle view, the foot pole has apical pore fields on each valve and the valvocopula has a single row of round poroids (Fig. 70). Internal views of the D-valve show troughs between the virgae (Fig. 71) as well as lineolate internal openings to the external areolae (Figs 71–74). Prominent pseudosepta are present at each pole (Figs 71, 73, 74) and raphe branches extend beyond the pseudosepta at each pole (Figs 71, 73). The proximal raphe ends are crook-shaped in the same direction (Figs 71, 73).

Type: USA. California: Ash Creek, Lassen County, 41.134220º N, 120.800250º W, collected by SWAMP Field Crew, September 15, 2010 (holotype ANSP! Circled specimen on slide GC 65219 made from ANSP GCM 5697, illustrated in Fig. 45; isotype JPK! 6204, slide and material, University of Colorado, Museum of Natural History, Kociolek Collection, Boulder, Colorado, USA).

Etymology: This species is named in honor of Dr. Rex L. Lowe, a good friend, teacher and mentor to the authors.

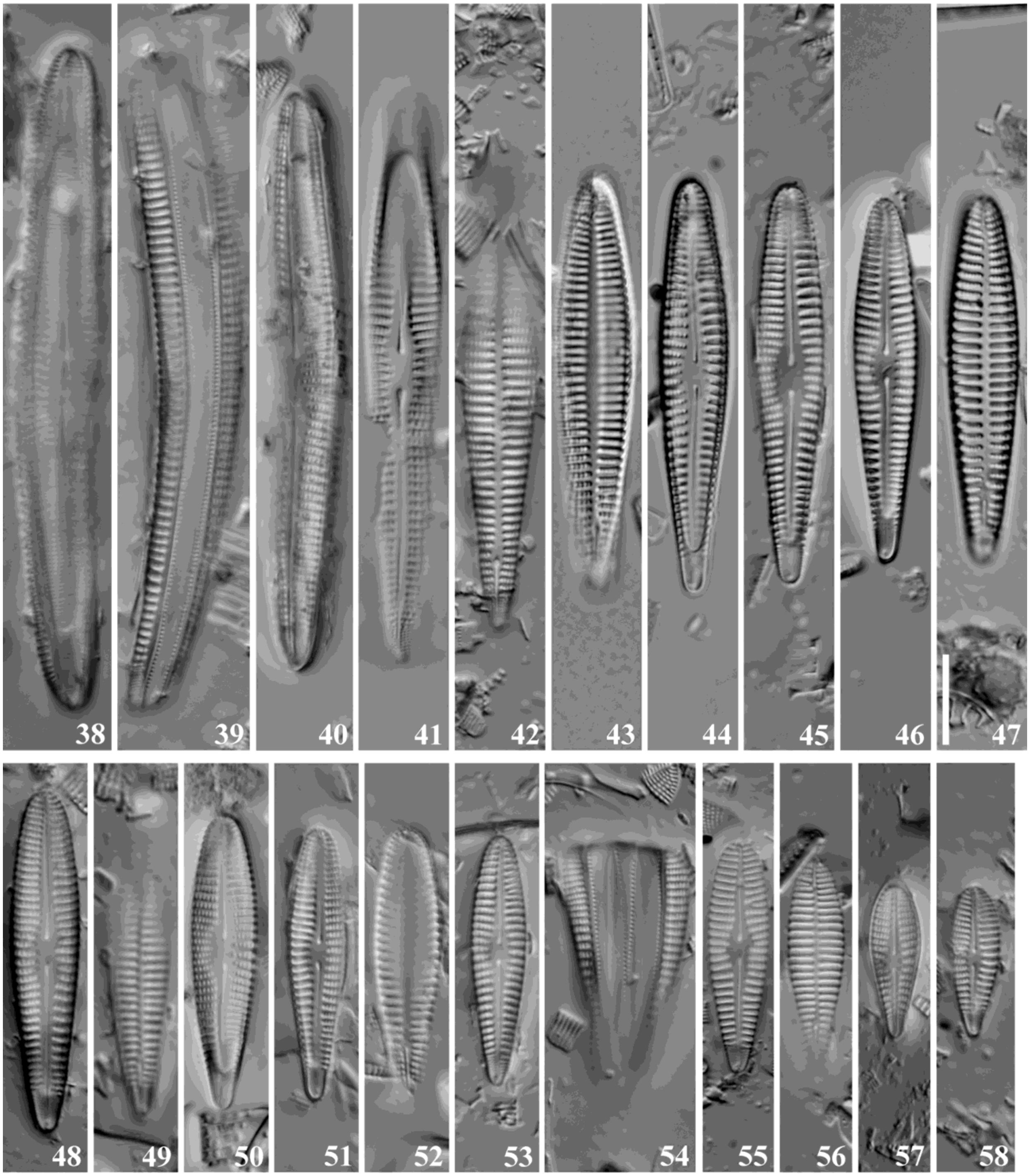
Taxonomic remarks: *Rhoicosphenia lowei* is distinguished from *R. stoermeri* by its smaller cardinal points of its size range; *R. lowei* has its smallest valve length of 16 µm and largest of 75 µm, while the cardinal points of the other large species in this paper, *R. stoermeri*, are 25 µm for the smallest and 84 µm for the largest valves. In addition, the shape of *R. lowei* is more linear with blunt apices, versus the lanceolate valves with acute apices of *R. stoermeri*. Of these two species, *R. lowei* has the coarsest striae, 9–11 in 10 µm, while the other, *R. stoermeri* has a higher density of, 11–13 in 10 µm. Finally, with regard to these two species, *R. lowei* has a shorter distance between proximal raphe ends. Both of these species share the larger panduriform central area, however it is less pronounced in *R. lowei*.

Compared to images of *R. abbreviata* in multiple publications through time (as *R. curvata*, Patrick & Reimer 1966, Pl. 20, Figs 1–5; Krammer & Lange-Bertalot 1986, Fig. 91, images 20–28; Levkov et al. 2010, Figs 1a–p), *R. lowei* can fit the broadest species concept of *R. abbreviata* in many aspects of its morphology. In terms of size, *R. lowei* is 16–75 µm long and 6–8 µm wide, and *R. abbreviata* has been reported to be 12–75 µm long and 4–8 µm wide (as *R. curvata*, Patrick & Reimer 1966), 10–75 µm long and 3–8 µm wide (Krammer & Lange-Bertalot 1986) 14–52 µm long and 5–7 µm wide (Levkov et al. 2010). However, when comparing *Rhoicosphenia lowei* to *R. abbreviata*, the large, oblanceolate to linear-clavate valve outline differentiates it from the linear to narrowly-clavate smaller valves of *R. abbreviata*. These two species have similar central areas and their striae densities overlap. In the SEM, *R. lowei* has C-shaped areolae along the axial area, which are not documented in *R. abbreviata* (Levkov et al. 2010, Figs 2c, 2e).

In valve shape, *R. lowei* is also somewhat similar to *R. marina* var. *intermedia* M. Schmidt (1899, Pl. 213, Figs 37–39), but the valves of *R. lowei* are narrower. The striae of *R. lowei* are less dense at 9–11 in 10 µm, while *R. marina* var. *intermedia* has 14 striae in 10 µm. In addition, *R. lowei* is a freshwater species and *R. marina* var. *intermedia* is reported from marine habitats. Finally, the species *R. lacustris* Levkov in Levkov et al. (2010, Figs 22a–x) is morphologically similar to *R. lowei*. Key differences can be found in shape, with the headpole of *R. lowei* being narrower than the bluntly rounded headpole of *R. lacustris,* size, the 16–75 µm long of *R. lowei* is greater than the 25–62 µm long of *R. lacustris,* however, no images of initial valves are provided in Levkov et al. (2010). In addition, the striae of *R. lacustris* are denser at

13–15 in 10 µm versus 9–11 in 10 µm of *R. lowei* and the areolar density is higher in *R. lacustris* at ~45 in 10 µm versus 30 in 10 µm in *R. lowei*. *Rhoicosphenia lowei* also has smaller septum like structures and pseudosepta, as well as a larger aperture in the pseudoseptum. Another key difference between the two species is habitat, with *R. lowei* being found in freshwater and *R. lacustris* being found in eutrophic freshwater to brackish environments.

Distribution and ecological notes: *Rhoicosphenia lowei* is found throughout the state of California from the Oregon border in the north to Los Angeles in the south, but more commonly reported north of Santa Cruz. It is found in samples across a wide range of elevations, from 11 to 2000 meters above sea level. Most locations are characterized by low conductivity, as well as low nitrogen and phosphorus.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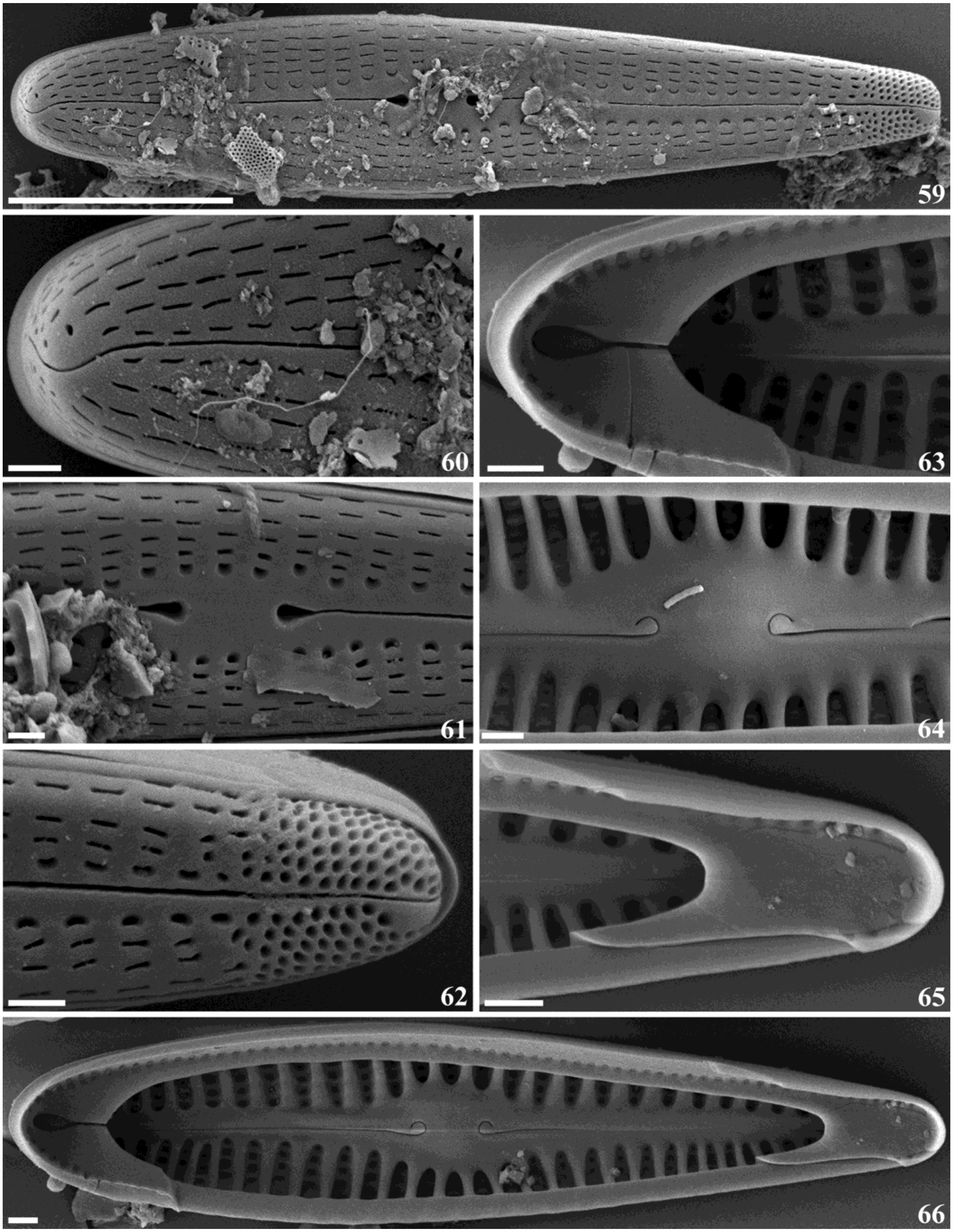
Figures 38–58: Type material of *Rhoicosphenia lowei* from Ash Creek, Lassen County, California, USA. LM. 45. Holotype specimen. 40, 41, 44–46, 48, 50, 51, 53, 55, 57, 58. R-valves. 42, 43, 47, 49, 52, 56. D-valves. 39, 54. Girdle views. 38. Post-auxospore. Scale bar is 10 μm.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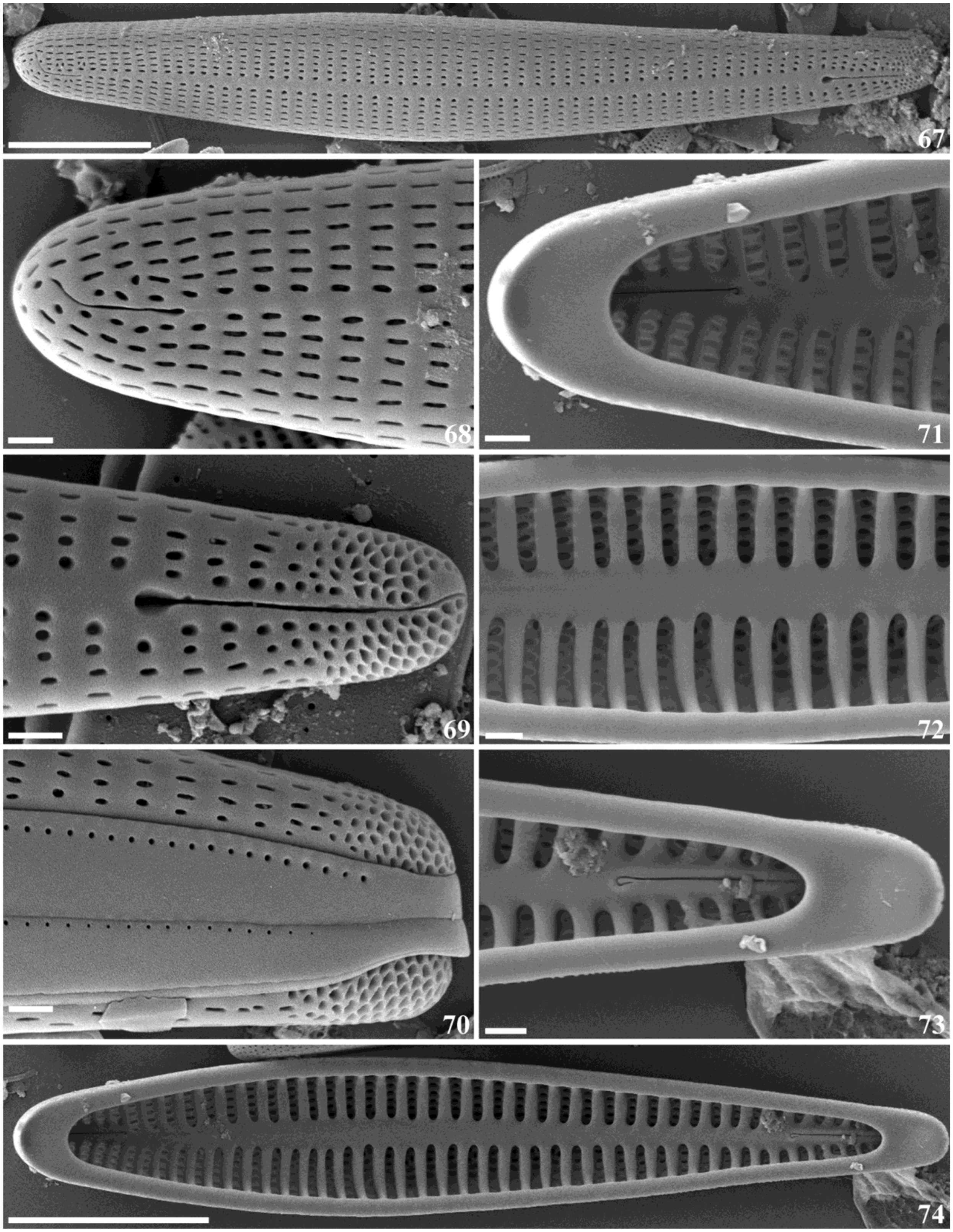
Figures 59–66: Type material of *Rhoicosphenia lowei* from Ash Creek, Lassen County, California, USA. SEM. 59–62. External views of R-valve show the bluntly rounded head pole, lineolate areolae, and dilated proximal raphe ends. The apical pore field has porelli that are more densely arranged, smaller, and rounder than the stria areolae (62). The distal raphe ends on R-valve continue onto the mantle (60, 62). 63–66. Internal R-valve shows the valvocopula is modified to overlap with the pseudosepta at each end of valve (63, 65, 66). The internal proximal raphe ends are strongly crook-shaped in the same direction (64). Scale bars are 10 µm (59), 1 µm (60–66).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Figures 67–74: Type material of *Rhoicosphenia lowei* from Ash Creek, Lassen County, California, USA. SEM. 67–70. The external of the D-valve is characterized by the shortened raphe branches and lineolate areolae (67). The distal raphe end on D-valve terminates on valve face and the head pole (68) and continues onto the mantle at the foot pole (69). The proximal raphe ends are dilated and drop-shaped (68–69). In girdle view, the foot pole has apical pore fields on each valve and the valvocopula has a single row of round poroids (70). 71–74. Internal views of the D-valve show troughs between the virgae (72) as well as lineolate internal openings to the external areolae (71–74). Prominent pseudosepta are present at each pole (71, 73, 74) and raphe branches extend beyond the pseudosepta at each pole (71, 73). The proximal raphe ends are crook-shaped in the same direction (71, 73). Scale bars are 10 μm (67, 74), 1 μm (68–73).

Rhoicosphenia californica **E.W. Thomas & Kociolek*, sp. nov.*** (Figs 75–110)

Frustules clavate and strongly flexed in girdle view. Valves heteropolar in valve view, linear-lanceolate with protracted apices in larger specimens and rounded apices in smaller specimens, 8–50 µm long, 3–6 µm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). R-valve: raphe filiform, proximal raphe ends dilated externally, crook-shaped internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area narrow, widening at small ovate central area. Striae parallel to radiate in center of the valve and radiate at apices, 11–12 striae in 10 µm at center of valve, 13–15 striae in 10 µm at apices, composed of round to lineolate areolae, 40 in 10 µm. D-valve: raphe branches 3–5 µm long at head pole, not extending beyond pseudoseptum, and 5–7 µm long at foot pole, external proximal ends not expanded, internal proximal ends crook-shaped in same direction and distal ends not inflated externally, terminate in helictoglossae internally. Striae parallel throughout, 12–14 striae in 10 µm at center of valve, 13–16 striae in 10 µm at apices, composed of round to lineolate areolae. Both valves with pseudosepta at each apex, 3–8 µm long. Both valves with apical pore field at foot pole, porelli 4 per 1 µm. Girdle bands open.

In the SEM, an external view of the R-valve shows rounded puncta near the axial area and lineate puncta towards the margins (Figs 98, 100). At the head pole, the raphe continues onto the mantle and an open girdle band is visible (Fig. 99). The central area has inflated proximal raphe ends (Figs 98, 100), and the foot pole has an apical pore field of rounded porelli (Fig. 101). Internal views show the areolae through troughs between the virgae (Figs 102–104) and the proximal raphe ends are strongly crook-shaped in the same direction (Figs 103–104). The valvocopula in modified to overlap the pseudosepta and an aperture is present in the valvocopula at the head pole (Figs 102, 104), but not at the foot pole (Fig. 104). External views of the D-valve show the shortened raphe branches and round to lineolate areolae (Fig. 105). The distal raphe end at the head pole terminates on the valve face (Fig. 106) and continues onto the mantle at the foot pole (Fig. 107). The proximal raphe end at the head pole is small (Fig. 106) are at the foot pole is dilated (Fig. 107). In girdle view, the foot pole can be seen on each valve along with a single row of simple poroids on the valvocopula (Fig. 108). Internal views show the external areolae through troughs between the virgae (Fig. 109). Pseudosepta are covered at each pole by the valvocopula and the raphe branches extend beyond the pseudosepta at each pole (Fig. 109). The proximal raphe end at the foot pole is crook-shaped and a small aperture in the valvocopula is evident (Fig. 109). In girdle view, the valvocopula are ornamented with a single row of simple poroids (Fig. 110).

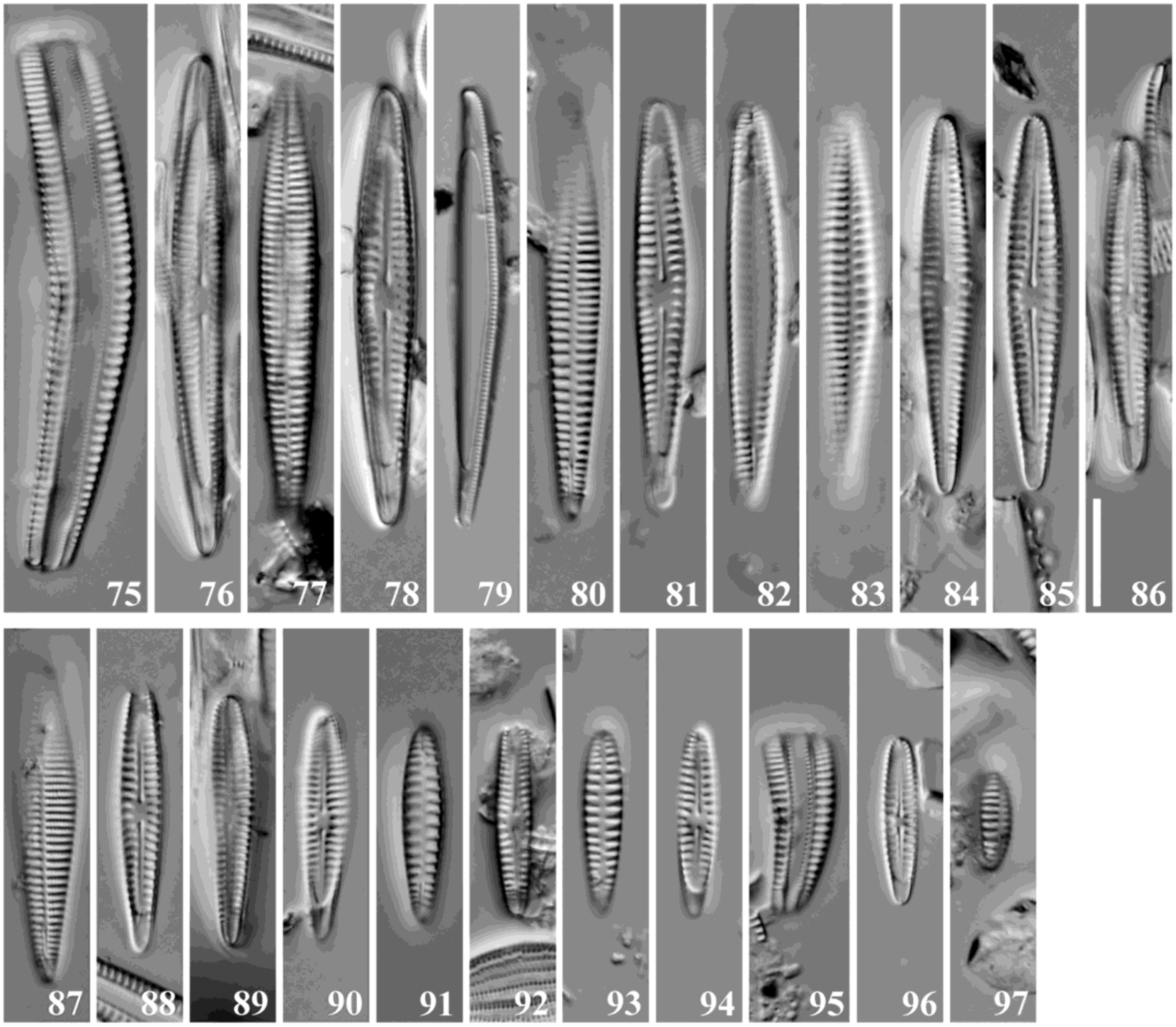
Type: USA. California: Big Chico Creek, Butte County, 39.72855º N, 121.88105º W, collected by SWAMP Field Crew, June 30, 2008 (holotype ANSP! Circled specimen on slide GC 65220 made from ANSP GCM 5698, illustrated in Fig. 84; isotype JPK! 3046, slide and material, University of Colorado, Museum of Natural History, Kociolek Collection, Boulder, Colorado, USA).

Etymology: Named for the state of California, where it commonly occurs.

Taxonomic remarks: *Rhoicosphenia californica* is the species most different from the other species described in this paper and is also distinct from all other previously described taxa. The long, narrow valves distinguish *R. californica* from other species in California, but outside of the state, several other *Rhoicosphenia* have a similar valve shape. *Rhoicosphenia tenuis* Levkov & Nakov (2008) is similar in length, but *R. californica* is wider (up to 6 µm) versus 5 µm in *R. tenuis*, and has less dense striae. Further, *R. tenuis* has very linear sides while *R. californica* is linear-lanceolate. *Rhoicosphenia tenuis* lacks an aperture in its valvocopula (Levkov et al. 2010, Fig. 14e), while *R. californica* has an aperture in its valvocopula (Figs 102, 109). *Rhoicosphenia californica* also resembles *R. baicalensis* Skabichevskii (1976) (Levkov et al. 2010, Figs 15a–y), but the shape of *R. californica* is more angular and less linear than *R. baicalensis* and has denser striae at 11–12 in 10 µm versus 9–12 in 10 µm. Finally, *R. californica* is linear-lanceolate and distinct from the narrowly lanceolate *Rhoicosphenia patrickae*. *R. californica* is wider at 6 µm versus 4.5 µm wide for *R. patrickae*, and has slightly denser striae, 11–12 in 10 µm versus 10–11 in 10 µm. In addition, *R. patrickae* does not have an aperture in the valvocopula (Thomas et al. 2015, Fig. 83) while *R. californica* does possess an aperture.

The linear-lanceolate valves with protracted apices of *R. californica* distinguish this species from *R. abbreviata*, which is linear to narrowly clavate, but does not have protracted apices (Levkov et al. 2010, Figs 1a–v). The striae of *R. abbreviata* are lineate on both valves (Levkov et al. 2010, Figs 2b, 2c, 2e, 2f, 3a–c), while the striae of *R. californica* are lineate, but the striae bordering the axial area are often rounded (Figs 98–101, 105–107).

Distribution and ecological notes: *Rhoicosphenia californica* is the most commonly and widely distributed *Rhoicosphenia* in the state of California. Found in streams from sea level to approximately 2700 meters across a wide range of freshwater conductivities and nutrient levels.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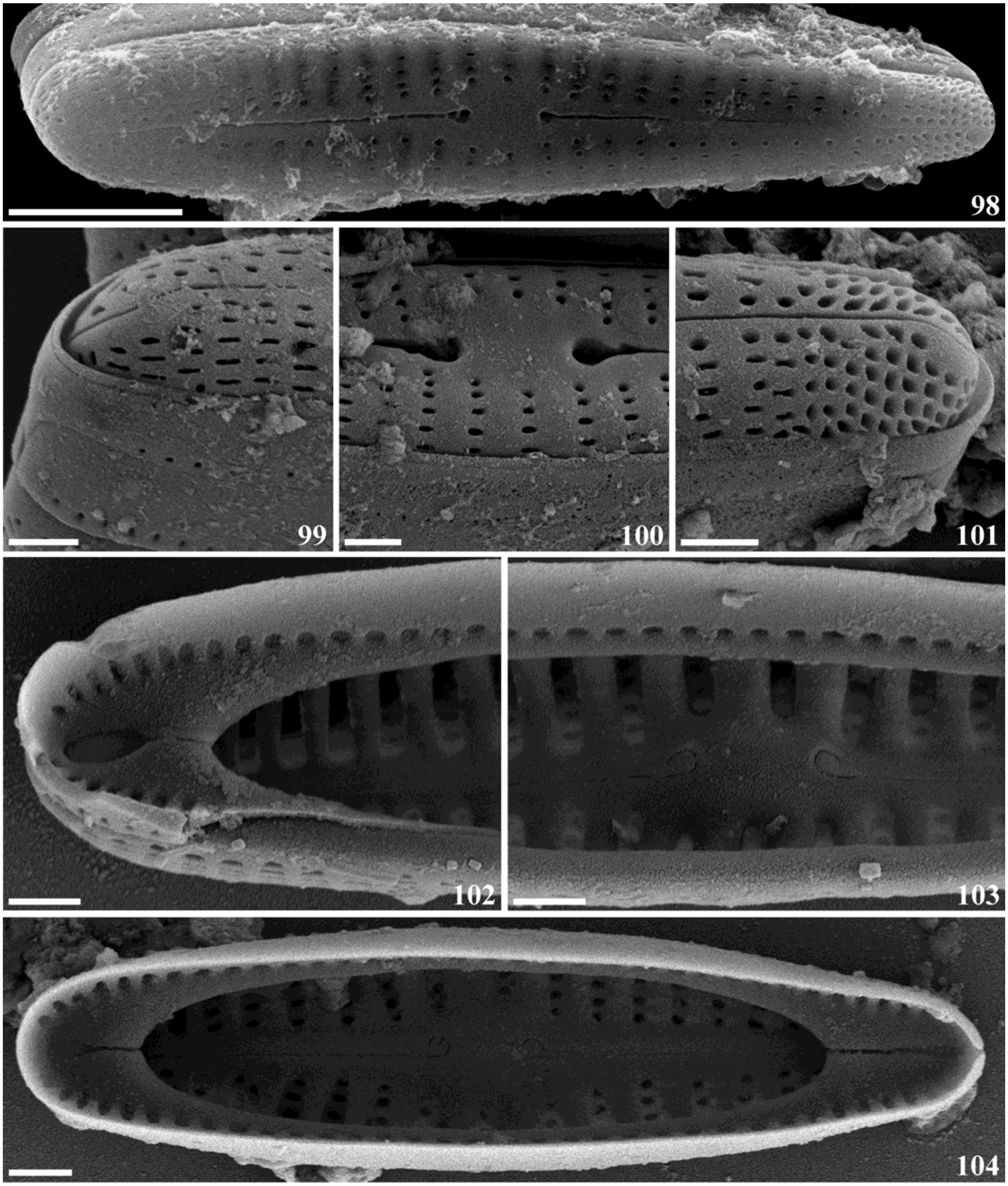
Figures 75–97: Type material of *Rhoicosphenia californica* from Big Chico Creek, Butte County, California, USA. LM. 84. Holotype specimen. 76, 78, 81, 84–86, 88–90, 92, 94, 96. R-valves. 77, 80, 82, 83, 87, 91, 93, 97. D-valves. 75, 95. Girdle views. 79. Valvocopula. Scale bar is 10 μm.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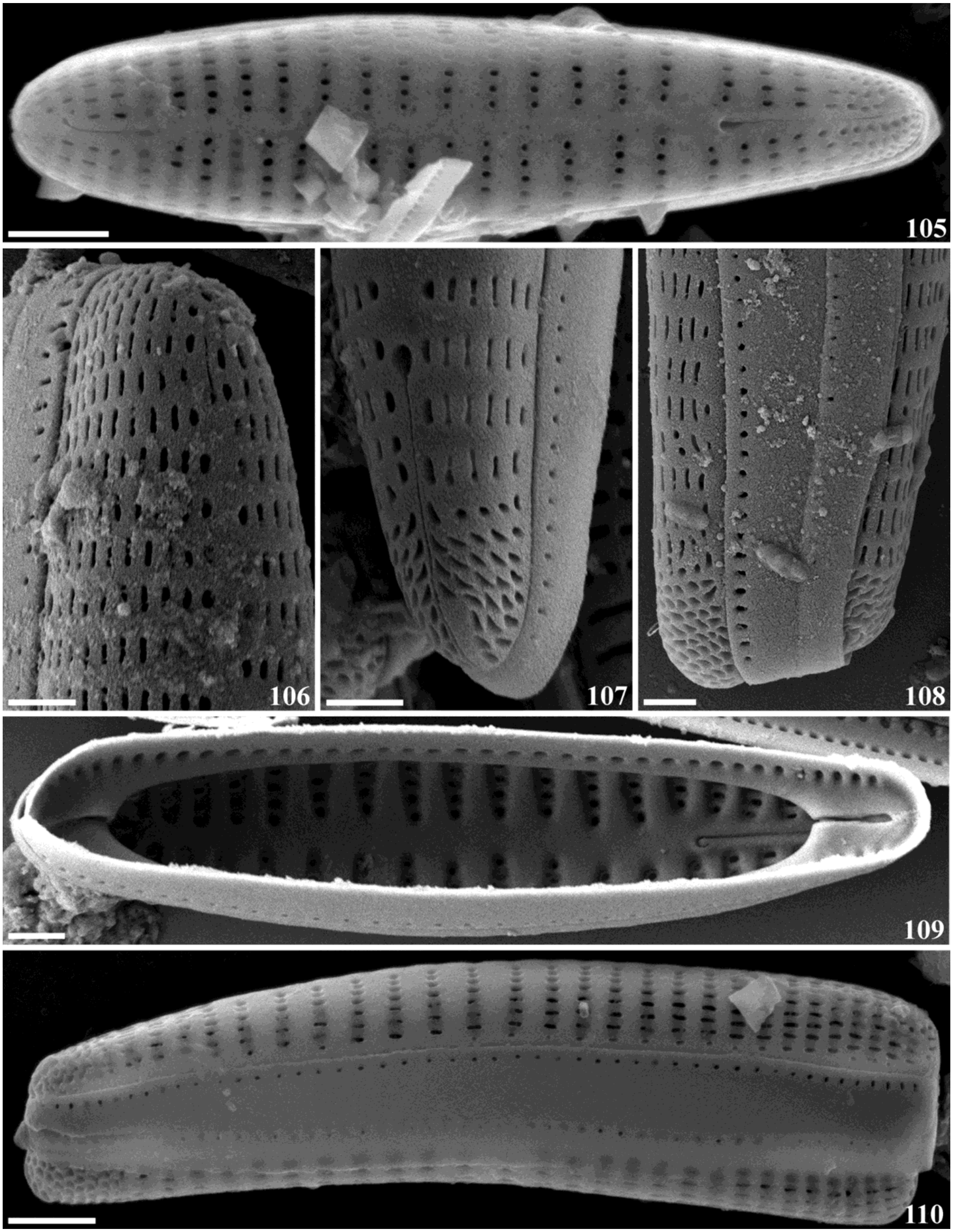
Figures 98–104: Type material of *Rhoicosphenia californica* from Big Chico Creek, Butte County, California, USA. SEM. 98–101. External views of the R-valve shows rounded puncta near the axial area and lineate puncta towards the margins (98, 100). At the head pole, the raphe continues onto the mantle and an open girdle band is visible (99). The central area has inflated proximal raphe ends (98, 100), and the foot pole has an apical pore field of rounded porelli (101). Internal views show the areolae through troughs between the virgae (102–104) and the proximal raphe ends are strongly crook-shaped in the same direction (103, 104). The valvocopula in modified to overlap the pseudosepta and an aperture is present in the valvocopula at the head pole (102, 104), but not at the foot pole (104). Scale bars are 5 µm (98) and 1 µm (99–104).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Figures 105–110: Type material of *Rhoicosphenia californica* from Big Chico Creek, Butte County, California, USA. SEM. 105–108. External views of the D-valve show the shortened raphe branches and round to lineolate areolae (105). The distal raphe end at the head pole terminates on the valve face (106) and continues onto the mantle at the foot pole (107). The proximal raphe end at the head pole is small (106) are at the foot pole is dilated (107). In girdle view, the foot pole can be seen on each valve along with a single row of simple poroids on the valvocopula (108). Internal views show the external areolae through troughs between the virgae (109). Pseudosepta are covered at each pole by the valvocopula and the raphe branches extend beyond the pseudosepta at each pole (109). The proximal raphe end at the foot pole is crook-shaped and a small aperture in the valvocopula is evident (109). In girdle view, the valvocopula are ornamented with a single row of simple poroids (110). Scale bars are 2 μm (105, 110), 1 μm (106–109).

Key to identify California *Rhoicosphenia* species and *Rhoicosphenia abbreviata*

1. Striae on both R- and D-valves are distinctly punctate … 2

- Striae on both R- and D-valves are not distinctly punctate … 3

2. On raphe valves proximal raphe ends close together, less than 5 µm apart; valves oblanceolate, striae 9–11 in 10 µm on both valves … *R. lowei*

- On raphe valves proximal raphe ends far apart, between 5–10 µm of separation … *R. stoermeri*

3. Valves linear-lanceolate, 3–6 µm wide, with narrow axial area and ovate central area … *R. californica*

- Valves linear to narrowly clavate, 5–7 µm wide, with wide central and axial area tapering to valve apices … *R. abbreviata*

Discussion

Rhoicosphenia morphology is distinct from other raphid diatoms as it is asymmetrical to the transapical axis, bent in girdle view, has pseudosepta and septum-like structures (Thomas et al. 2015), complete raphe branches on one valve, and shortened raphe branches on the other valve. Nearly all *Rhoicosphenia* species (with the exception of *R. genuflexa*, which is symmetrical to the transapical axis) share these characters and interspecific variation is often seen in valve size, shape and striae arrangement and density. Thus, valve morphological features that diagnosis the genus are distinct and easy to recognize with light microscope.

A few potential explanations for the lack of recently described new species may exist, mainly due to the lack of prominent features. First, when identifying or enumerating diatoms, a diatom bent in girdle view and possessing a distinct head and foot pole, is most likely a *Rhoicosphenia*. Second, the nature of the bent valves does not allow them to be oriented in valve view for detailed investigation. In samples where the relative abundance of *Rhoicosphenia* is less than 10% it can be challenging to find many individuals in valve view (personal observation). These two problems make it difficult to assess the valve characters, such as shape and striation, which are often most critical to differentiate species and therefore, often resulting in a 'default' identification of the individual as the 'cosmopolitan' *R. abbreviata*. Until recently there have been relatively few species in the literature with detailed photomicrographs, as opposed to older line drawings in more obscure literature. Commonly used literature show broad morphologies attributed to *R. abbreviata* (Krammer & Lange-Bertalot 1986, Potapova 2009). Finally, there has been the notion that the species *R. abbreviata*, is cosmopolitan and tolerant of broad ranges of ecological conditions (Bahls 2009, Johansen et al. 2007, Lowe 1970, Potapova 2009, ANS 2011–2016). The cumulative effect of all of these factors is that *Rhoicosphenia* is lumped into few (or one), common species.

Of the 62 previously described *Rhoicosphenia*, approximately half were described prior to 1900 (Fourtanier & Kociolek 2011) and only eleven have been described recently enough to have scanning electron micrographs included with their initial descriptions (Levkov et al. 2007, 2010, Levkov & Nakov 2008, Thomas et al. 2015). The majority of early descriptions were done by M. Schmidt (1899; 12 taxa), Kützing (1833, 1844, 1849; 7 taxa), and Cleve-Euler (1915, 1932, 1953; 6 taxa), while Levkov and colleagues have described the only new extant species (Levkov et al. 2007, 2010, Levkov & Nakov 2008; 7 taxa) since 1980. The year 1980 marked an important historical point in the study of *Rhoicosphenia* due to the publishing of a manuscript that suggested *R. abbreviata* and *R. curvata* are synonyms (Lange-Bertalot 1980). The effect of this proposal has been long lasting in that it broadened the morphological species concept of *R. abbreviata* (*sensu* Krammer & Lange-Bertalot 1986). Only recently has anyone suggested that the infraspecific taxa of *R. curvata* described from non-freshwater habitats and possessing different morphologies making their conspecificity with *R. curvata* (= *R. abbreviata*) dubious (Levkov et al. 2010). One report on the flora of North American diatoms reports that *R. abbreviata* is likely the only extant species in North America (Kociolek & Spaulding 2003).

The discovery of these three new species of *Rhoicosphenia* in California contradicts a centuries worth of records indicating that the diversity of extant members of this genus is low in the United States (Patrick & Reimer 1966, Czarnecki & Blinn 1978, Kociolek 2005). One reason these new species discoveries is so striking is that California represents 5% of the contiguous US land area, and approximately 4% of European land area, and these new species now account for approximately 5% species of globally described *Rhoicosphenia* taxa. These results also highlight the need for biodiversity research in well-studied taxa (Ceballos & Ehrlich 2008) and from well-studied locations (Harris & Froufe 2004, Tripp & Lendemer 2012). Further, an increased taxonomic resolution has the potential to enhance freshwater conservation efforts (Cook et al. 2008) and highlights the importance of morphology-based alpha taxonomy (Schlick-Steiner et al. 2007). Also, these taxa may provide insight into the current debate surrounding microbial eukaryotes, endemism, and cosmopolitanism (Williams & Reid 2006) as they all vary in their geographical ranges, as well as niche requirements. For many years following the “everything is everywhere” hypothesis (Baas-Becking 1934), in regards to microbial distributions, free-living microbial eukaryotes, including diatoms have been thought to have global distributions (Finlay 2002). However, other analyses of diatoms have produced results contrary to the “everything is everywhere” hypothesis (Kociolek & Spaulding 2000, Telford et al. 2006, Theriot et al. 2006) and the newly described diversity of *Rhoicosphenia* in California may also provide results contrary to that hypothesis.

Two of the new species, *Rhoicosphenia stoermeri* and *R. lowei*, are large. Of the previously described taxa, only *Rhoicosphenia curvata* var. *subacuta* Schmidt and *Rhoicosphenia curvata* var. *major* Cleve (1895) have individuals greater than 70 µm in length, while both of these species are described from populations with individuals longer than 75 µm in length. In addition, *R. stoermeri* has proximal raphe ends that are up to 10 µm apart in larger specimens, a feature not found in other *Rhoicosphenia*. One species morphologically similar to *R. stoermeri* is *Rhoicosphenia affinis* Levkov, and is found in China, geographically distant from California, and the ecology of *R. affinis*, found in eutrophic waters, distinguishes it from the less eutrophic streams that *R. stoermeri* inhabits. Some species in the diatom genus *Gomphosinica* Kociolek et al. (2015a), are known to have disjunct distributions in the western US and Asia. The distributions of *Gomphosinica* as well as *R. stoermeri* and *R. affinis* support a hypothesis that some diatoms in the western US are more similar to species ('forms') in China, than they are to species in the eastern US due to the barrier of the Rocky Mountains (Ehrenberg 1849). Although the morphological diversity of *Rhoicosphenia* is understudied in the eastern US, preliminary personal observations suggest that large taxa are not found east of the Rocky Mountains. *Rhoicosphenia lowei* is morphologically similar to *R. lacustris* Levkov, but the former is found in freshwaters (conductivity 78.7–1142.0 µS/cm), while the latter is found in "freshwater to brackish" (Levkov et al. 2010) water habitats.

The other new species, *R. californica*, is most likely to be lumped into *Rhoicosphenia abbreviata* due to some overlapping size and striae density features from the broad description of *R. abbreviata*. Many diatom floristic publications show a variety of morphologies attributed to *R. abbreviata* from Europe (Krammer & Lange-Bertalot 1986, Levkov et al. 2010) and North America (as *R. curvata*, Patrick & Reimer 1966, Reavie & Smol 1998) as well as for *R. curvata* in NA (Boyer 1927, Benson & Rushforth 1975, Clark & Rushforth 1977, Czarnecki & Blinn 1977, 1978, Lawson & Rushforth 1975, Fungladda et al. 1983, Kaczmarska & Rushforth 1983) and also report a wide range of ecological parameters that these populations were found to occur in. Size ranges for the European populations of *R. abbreviata* are 10–75 µm long, 3–8 µm wide, with 9–12 striae in 10 µm at the center of the R-valve (Krammer & Lange-Bertalot 1986, Levkov et al. 2010), and 12–75 µm long, 4–8 µm wide, with 9–15 striae in 10 µm at the center of the R-valve (as *R. curvata*, Patrick & Reimer 1966; Reavie & Smol 1998) for North American populations. Similarly wide are the ranges of morphological statistics for *R. curvata* in NA with a cumulative size range of 12–75 µm long, 3–10 µm wide, with 8–19 striae in 10 µm at the center of the R-valve (Boyer 1927, Benson & Rushforth 1975, Lawson & Rushforth 1975, Clark & Rushforth 1977, Czarnecki & Blinn 1977, 1978, Fungladda et al. 1983, Kaczmarska & Rushforth 1983). It is likely that these wide ranges of form and niche represent several undescribed *Rhoicosphenia* species from the North American flora.

Finally, these new *Rhoicosphenia* species should not be viewed as examples of cryptic or pseudocryptic diversity as they are different in several morphological and ecological features and therefore do not fit the definitions of crypsis nor pseudocrypsis. Cryptic species, which may hide diversity, have been studied in many taxonomic groups; mammals (Brown et al. 2007), insects (Molbo et al. 2003), vascular plants (Whittall et al. 2004, Okuyama & Kato 2009), brown algae (Fraser et al. 2009), and diatoms (Mann et al. 2004). Similarly, the concept of pseudocryptic species is used to describe morphological differences undetected until another technique, often molecular data, suggest that two morphologically similar species may be more different than previously observed by morphology alone (Amato & Montresor 2009, Vanelslander et al. 2009). In the case of *Rhoicosphenia* species discovery and taxonomy in the United States and globally, we argue that neither cryptic nor pseudocryptic species are the problem. Rather, a broad species concept, lack of high quality photomicroscopic documentation, and an assumption of a cosmopolitan distribution for *R. abbreviata* seem to be responsible for the lack of species descriptions within the genus in the United States. Future investigations into the freshwater diversity of *Rhoicosphenia* are likely to uncover additional undocumented diversity and will be used to further analyze the relationship between *Rhoicosphenia* species, biogeography, and ecological characteristics of the habitats in which they are found.

Acknowledgements

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Four new *Rhoicosphenia* species from fossil deposits in India and North America

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Abstract

Rhoicosphenia Grunow is a common diatom in both freshwater and marine ecosystems and the genus can be found on nearly every continent. Most of the presently described taxa are extant and only 6 of 58 taxa are known from the fossil record. Also, reports from freshwater habitats of the Indian sub-continent are rare, with one report of *R. marina* from marine ecosystems. *Rhoicosphenia* is common in freshwater and marine ecosystems of the United States and three fossils have been described from the Pacific Northwest. Four new species of *Rhoicosphenia* are described from fossil deposits in Gujarat, India and Oregon, USA. The new species from India are *R. gandhii*, a large, coarsely ornamented species and *R. indica*, a smaller species, both found in the same fossil deposit. The species from Oregon are *R. reimeri*, another large taxon, as well as *R. patrickae*, another small species, and both are found in the same fossil deposit. These new species descriptions highlight the recent trend of discovery of *Rhoicosphenia* diversity over the past decade. Finally, a discussion of the valvocopula structure in *Rhoicosphenia* is included with comparisons to septa and septum-like structures of other diatom genera.

Keywords: *Rhoicosphenia*, fossils, India, Oregon, valvocopula, septum

Introduction

Rhoicosphenia Grunow is a common genus in freshwater (Czarnecki & Blinn 1977, Levkov et al. 2007) and marine (Hällfors 2004, Harper et al. 2012, Misra 1956) ecosystems and many extant taxa are found globally. Currently, there are 28 *Rhoicosphenia* species and approximately 30 intraspecific *Rhoicosphenia* taxa included in the California Academy of Sciences on-line Catalogue of Diatom Names (Fourtanier & Kociolek 2011). Of the 58 described taxa, only six have been described from fossil material, three fossil taxa from western North America and three fossil taxa from Europe (Cleve 1895, Schmidt 1899, Cleve-Euler 1915, 1953). While reports of fossil and extant *Rhoicosphenia* in North America and Europe are common, there have been very few reports from India. To date, no fossil *Rhoicosphenia* have been described from Asian material. Also, there are very few reports of *Rhoicosphenia* in India, with one report of a marine taxon (Misra 1956). Presently, extant *Rhoicosphenia* is known to be globally distributed and the new reports from Indian fossils suggest that the genus may have been widely distributed in the past as well.

Rhoicosphenia is not only common in many ecosystems, but also has a distinctive frustule morphology that leads to rapid genus-level identification. Some of these distinct morphological characters include the concave R-valve with a complete raphe, convex D-valve with shortened raphe branches, frustule flexed in girdle view, and asymmetry about the transapical axis (with few exceptions in this character). In the 1980's, several studies examined and documented features of *Rhoicosphenia* vegetative valve morphology, auxospore formation, and phylogeny using three different species, *R. curvata*, *R. adolfi*, and *R. genuflexa* (Mann 1982a, Mann 1982b, Medlin & Fryxell 1984a, Medlin & Fryxell 1984b). These studies provided detailed information on the valve structure, especially in regards to the R-valve and D-valve and how they relate to phylogeny (Mann 1982a). Details on the ecological characteristics of habitats in which *Rhoicosphenia* species are found are usually lacking and are often given as statements about having little to no affinities with specific conditions (Czarnecki & Blinn 1977, 1978, Foged 1984b). However, one large-scale study has shown a positive relationship between *Rhoicosphenia* and high nutrients (Potapova & Charles 2007).

Over the past decade, investigations into *Rhoicosphenia* diversity have increased resulting in the description of new species (Levkov et al. 2007, 2010, Levkov & Nakov 2008). These studies of *Rhoicosphenia* have re-examined the known common, widely reported species, and described new taxa (Levkov et al. 2007, 2010, Levkov & Nakov 2008). However, no new fossil taxa were described and none of the recently described species are from North America. Our investigation of fossil material from both India and the United States has yielded four previously undescribed species of *Rhoicosphenia*, nearly doubling the number of known extinct species within the genus. The present report offers detailed descriptions of these four species based on light and scanning electron microscope observations of valve and girdle elements, and discusses girdle band characters that may prove useful in phylogenetic reconstructions. In past discussions of *Rhoicosphenia* ultrastructure, the septum-like structure on the valvocopula was often ignored (Mann 1982a, Mann 1982b, Medlin & Fryxell 1984a, Levkov et al. 2007, 2010, Levkov & Nakov 2008). This feature has been referred to as a modified valvocopula (Levkov et al. 2010) and various aspects of its structure and evolutionary implications are discussed.

Materials and Methods

The type material of the Indian species was collected by H.P. Gandhi from Galteshwar near Mahi River, Gujarat, India (21.28 N, 73.08 E), in October 1957. The Oregon (Northwestern United States of America) sample was collected by M.C. Whiting in July 1982. This fossil deposit is near Lower Bridge on the Deschutes River (44.359 N, 121.294 W). Slides and material for both samples are accessioned at the Academy of Natural Sciences, Philadelphia, Pennsylvania, as well as the University of Colorado, Museum of Natural History, Kociolek Collection, Boulder, Colorado. A summary of the type material can be found in Table 4.

ANS Accession #	**Location**	**Latitude**	**Longitude**	**Date Collected**	**Collector**
36349	Mahi River, Gujarat, India	21.28 N	73.08 E	October 1957	H.P. Gandhi
65211	Lower Bridge on Deschutes River, OR, USA	44.359 N	121.294 W	July 1982	M.C. Whiting

Table 4: Type locations for new fossil species.

Diatom samples were boiled in nitric acid, settled and rinsed with filtered water until pH was neutral, air dried onto cover glasses, and permanently mounted in Naphrax®. Light microscopy was performed using an Olympus® BX51 Photomicroscope (Olympus America Inc., Center Valley, Pennsylvania) with differential interference contrast optics. Specimen images were captured at 432 pixels/inch with an Olympus® DP71 Digital Camera attached to the Olympus® BX51 and a computer. Scanning electron microscopy (SEM) was performed with cleaned specimens air dried onto cover glasses, attached to aluminum stubs, sputter-coated with 5 nm of gold-palladium and examined in high vacuum mode using a JEOL JSM 6480LV low vacuum SEM (JEOL Ltd, Tokyo, Japan) with an accelerating voltage of 15 kV and a JEOL JSM 7401 field emission SEM (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 5 kV. SEM was performed at the Nanomaterials Characterization Facility, University of Colorado, Boulder. All images in this paper are from the type material. Terminology for the valves and copulae of

Rhoicosphenia follows Ross et al. (1979), Cox & Ross (1981), Mann (1982), and Levkov et al. (2010).

Results

***Rhoicosphenia gandhii* E.W. Thomas, B. Karthick & Kociolek sp. nov.**

Frustules clavate and very slightly flexed in girdle view. Valves heteropolar in valve view, oblanceolate with bluntly rounded apices, 16–48 µm long, 6.0–8.5 µm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). R-valve raphe filiform, proximal raphe ends expanded externally, recurved in same direction internally, distal raphe ends curved in same direction externally, ending in helictoglossae internally. Axial area narrow throughout, tapering at apices, central area small and lanceolate. Striae strongly radiate at center of the valve and slightly radiate throughout, 12–14 in 10 µm, composed of lineolate areolae, 30 in 10 µm. D-valve raphe 2–3 µm long at head pole, not extending beyond pseudoseptum, and 3–5 µm long at foot pole, internal proximal ends recurved in same direction and distal ends not inflated externally, terminating in helictoglossae internally. Striae parallel to slightly radiate in center, radiate at apices, 12–14 in 10 µm, composed of lineolate areolae. Both valves with 2–3 µm long pseudoseptum at each apex. Both valves with apical pore field at foot pole, porelli 4 per 1 µm. Girdle bands open.

In the SEM, external views of R-valve show lineolate areolae. Proximal raphe ends on R-valve are dilated and drop-shaped, as seen in LM. Distal raphe end on R-valve continues onto mantle. Apical pore fields are present only at foot pole and porelli are more densely arranged, smaller, and rounder than the stria areolae. Valvocopula with an aperture, interlocking with pseudoseptum at each end of valve. The valvocopula has simple, round poroids externally and internally.

External views of the D-valve show the shortened raphe and lineolate areolae. Distal raphe end on D-valve terminates on valve face. Proximal raphe ends on D-valve are dilated and drop-shaped. Internal SEM of the D-valve shows the areolae opening in a trough. Prominent pseudosepta are present at each pole and raphe branches extend beyond the pseudosepta at each pole. The valvocopula is modified to fit over the pseudoseptum, with a flange-like pars interior (indicated with arrow) following the valve interior, a feature not often documented with SEM in *Rhoicosphenia*, but shown by Mann (1982a, Fig. 46). The interior view of the foot pole has a recurved internal proximal raphe end.

Holotype: Circled specimen on slide ANSP GC 36349 made from sample ANSP GCM 24051, Academy of Natural Sciences, Philadelphia, USA.

Isotype: Slide and material JPK 4503, University of Colorado, Museum of Natural History, Kociolek Collection, Boulder, Colorado, USA.

Type locality: Galteshwar, near Mahi River, Gujarat, India. 21.28 N, 73.08 E, collected in October 1957 by H.P. Gandhi.

Taxonomic remarks: The shape, coarse ornamentation, and heavy silicification of *R. gandhii* are sufficient to distinguish it from other members of the genus. The oblanceolate shape of *R. gandhii* distinguishes it from *R. reimeri* that is lanceolate-clavate in shape. *Rhoicosphenia gandhii* also has a higher stria density of 12–14 striae in 10 µm versus 9–11 striae in 10 µm in *R. reimeri*. *Rhoicosphenia curvata* var. *subacuta* M. Schmidt is the *Rhoicosphenia* species most similar in shape, but lacks the lanceolate central area of *R. gandhii*. *Rhoicosphenia gandhii* and *R. curvata* var. *subacuta* overlap in both length and breadth, as well as stria density. However, this is most likely due to Schmidt's broad circumscription of the species accounting for specimens from distant, contrasting locations, including a recent marine sample from China

(‘Insel Hainan’) (Schmidt 1899, Pl. 213, Figs 6, 7), fossil freshwater from Mexico (Schmidt 1899, Pl. 213, Fig. 8) and Washington County, Oregon (Schmidt 1899, Pl. 213, Fig. 19), recent brackish Caspian Sea (Schmidt 1899, Pl. 213, Figs 9, 10) and Vancouver Island (Schmidt 1899, Pl. 213, Figs 11–14). Even comparing only the fossils depicted by Schmidt for *R. curvata* var. *subacuta* (Schmidt 1899, Pl. 213, Figs 8, 19), we find distinct differences in shape with the Mexican fossil (Schmidt 1899, Pl. 213, Fig. 8), which has a linear shape and the Oregon fossil (Schmidt 1899, Pl. 213, Fig. 19), which is lanceolate. If we compare *R. gandhii* to the geographically proximate Chinese specimens of *R. curvata* var. *subacuta* (Schmidt 1899, Pl. 213, Figs 6, 7), differences in stria count and shape are still present, with *R. gandhii* having 12–14 striae in 10 µm (versus 9–11 in 10 µm in the Chinese specimens) and more bluntly rounded apices.

Distribution: Known only from type locality.

Etymology: Named in honor of Professor H.P. Gandhi, one of the foremost Indian diatomists without whose samples this species would remain undescribed.

Rhoicosphenia indica **E.W. Thomas, B. Karthick & Kociolek sp. nov.**

Frustules clavate and strongly flexed in girdle view. Valves heteropolar, narrowly oblanceolate, with acute to narrowly rounded apices, 12.5–44.0 µm long, 3–4 µm wide. Frustules heterovalvate, one valve concave with elongated raphe (R-valve), one valve convex with shortened raphe branches (D-valve). R-valve raphe straight, proximal raphe ends expanded externally, recurved internally in same direction, distal raphe ends curved in same direction externally, ending in helictoglossae internally. Axial area narrow, expanded to rectangular central area. Striae parallel to radiate throughout, 11–13 in 10 µm, composed of lineolate areolae, 30 in 10 µm. D-valve raphe restricted to 1–2 µm at head pole and 3–4 µm at foot pole, internal proximal ends recurved in same direction and proximal ends not inflated externally, distal ends terminating in helictoglossae internally. Reduced raphe branches reach end of pseudoseptum at head pole, just beyond pseudoseptum at foot pole. Striae parallel to slightly radiate throughout, fine, appearing distinct from adjacent row, can be more radiate at apices, 11–14 striae in 10 µm. Both valves with 2–4 µm long pseudoseptum at each apex. Both valves with apical pore field at foot pole, porelli 40 in 10 µm. Girdle bands open.

As observed in the SEM, the exterior of the R-valve is characterized by lineolate areolae, with some rounded areolae beside the axial area. External proximal raphe ends on the R-valve are expanded and round. Distal raphe ends extend onto mantle at both poles and the apical pore field is bisected by the raphe. Apical pore field porelli are round, densely packed, 40 in 10 µm. Internally, the R-valve pseudosepta are covered by the valvocopula. Internal proximal raphe ends are recurved.

The exterior of the D-valve is characterized by lineolate areolae, some of which are rounded beside the axial area. The apical pore field is bisected by the shortened raphe at the foot pole and the apical pore field porelli contrast with the lineolate areolae of valve. Raphe at head pole is very short, 1–2 µm long, distal end does not extend on to mantle and proximal raphe end is expanded. The valvocopula has a row of simple poroids, and extensions of the valvocopula obscure the pseudosepta. The areolae can be seen in troughs between the virgae. Pseudosepta are present at both poles. The shortened raphe does not extend past the pseudoseptum at the head pole, but the raphe fissure extends beyond the pseudoseptum at the foot pole. The proximal end is recurved.

Holotype: Circled specimen on slide ANSP GC 36349 made from the sample ANSP GCM 24051, Academy of Natural Sciences, Philadelphia, USA.

Isotype: Slide and material JPK 4503, University of Colorado, Museum of Natural History, Kociolek Collection, Boulder, Colorado, USA.

Type locality: Galteshwar, near Mahi River, Gujarat, India. 21.28 N, 73.08 E, collected in October 1957 by H.P. Gandhi.

Taxonomic remarks: *Rhoicosphenia indica* can be distinguished from *R. tenuis* Z. Levkov & T. Nakov (Levkov & Nakov 2008) by its more acute valve apices and coarser striae. Also, *R. tenuis* has a valvocopula with an aperture, whereas the valvocopulae of *R. indica* do not have apertures. *Rhoicosphenia indica* differs from *R. fossilis* Cleve-Euler (Cleve-Euler 1953 Fig. 601 A) in length to breadth ratio, shape, density of striae; *R. indica* is longer and narrower than *R. fossilis*, has 12–13 striae in 10 μm (as opposed to 10 in 10 μm), and less strongly radiate striae. Also, the narrowly oblanceolate shape of *R. indica* is distinct from the lanceolate shape of *R. fossilis*. Although *R. fossilis* is only known from one line drawing (Cleve-Euler 1953 Fig. 601 A), it remains distinct from *R. indica* based on Cleve-Euler's interpretation. *Rhoicosphenia fossilis* is also geographically distant from the Indian *R. indica*, as it is in northwestern Russia, near Finland, a polar as opposed to a tropical diatom.

Distribution: Known only from type locality.

Etymology: Named for the country in which it was discovered.

Rhoicosphenia reimeri **E.W. Thomas & Kociolek sp. nov.** (Figs 111–133)

Frustules clavate and slightly flexed in girdle view. Valves lanceolate, becoming oblanceolate in smaller specimens, with drawn out and bluntly rounded apices, 18–70 μm long, 7–10 µm wide. Frustules heterovalvate, one valve concave with elongated raphe (R-valve), one valve convex with shortened raphe branches (D-valve). R-valve raphe filiform, proximal raphe ends expanded externally, recurved internally in same direction, distal raphe ends curved in same direction externally, ending in helictoglossae internally. Axial area narrow, expanding to form a rectangular central area, sometimes appearing panduriform in larger specimens. Striae strongly radiate in center of the valve, becoming parallel to slightly convergent at apices, 9–11 in 10 µm, composed of lineolate areolae, 30 in 10 µm. D-valve raphe 3–5 µm long at head pole and 4–8 µm long at foot pole, internal proximal ends recurved in same direction and distal ends not inflated externally, terminating in helictoglossae internally. Reduced raphe branches reach end of pseudosepta, extending beyond pseudoseptum at foot pole, striae composed of lineolate areolae, parallel throughout, slightly radiate at apices, 9–11 striae in 10 µm. Both valves with prominent pseudosepta at each apex, 6–8 µm long. Both valves with apical pore field at foot pole, porelli 30 in 10 µm. Girdle bands open.

As observed in the SEM, the exterior of the R-valve has lineolate striae, large drop-shaped proximal raphe ends, distal raphe ends curved in the same direction, and condensed, and rounded porelli in the apical pore fields (Fig. 124). Internally, lineolate exterior openings are visible in troughs between the virgae (Fig. 125). Prominent pseudosepta obscure helictoglossae at each pole (Fig. 125). The pseudoseptum is visible through an aperture in the valvocopula (Fig. 126). Girdle view of foot pole shows distinct difference between valve areolae and porelli of apical pore fields (Fig. 127). Internally, images of the D-valve show prominent pseudoseptum, recurved proximal raphe end at the foot pole, the areolae can be seen in troughs between the virgae, and lineolate external areolae (Fig. 128).

Externally, the D-valve has lineolate areolae and condensed, rounded porelli forming an apical pore field (Fig. 129). The head pole raphe branch is very short with a slightly inflated proximal end (Fig. 130). The raphe branch at the foot pole has more prominently expanded, drop-shaped proximal ends and the raphe bisects the apical pore field and continues onto mantle (Figs 129, 131, 133). In girdle view, the valve is weakly flexed, and the long, narrow lineolate areolae are evident. Each element of the cingulum bears a single row of simple poroids (Fig. 132).

Holotype: Circled specimen on slide ANSP GC 65211 made from material ANSP GCM 5689, Academy of Natural Sciences, Philadelphia, USA. Holotype specimen illustrated in Figure 52.

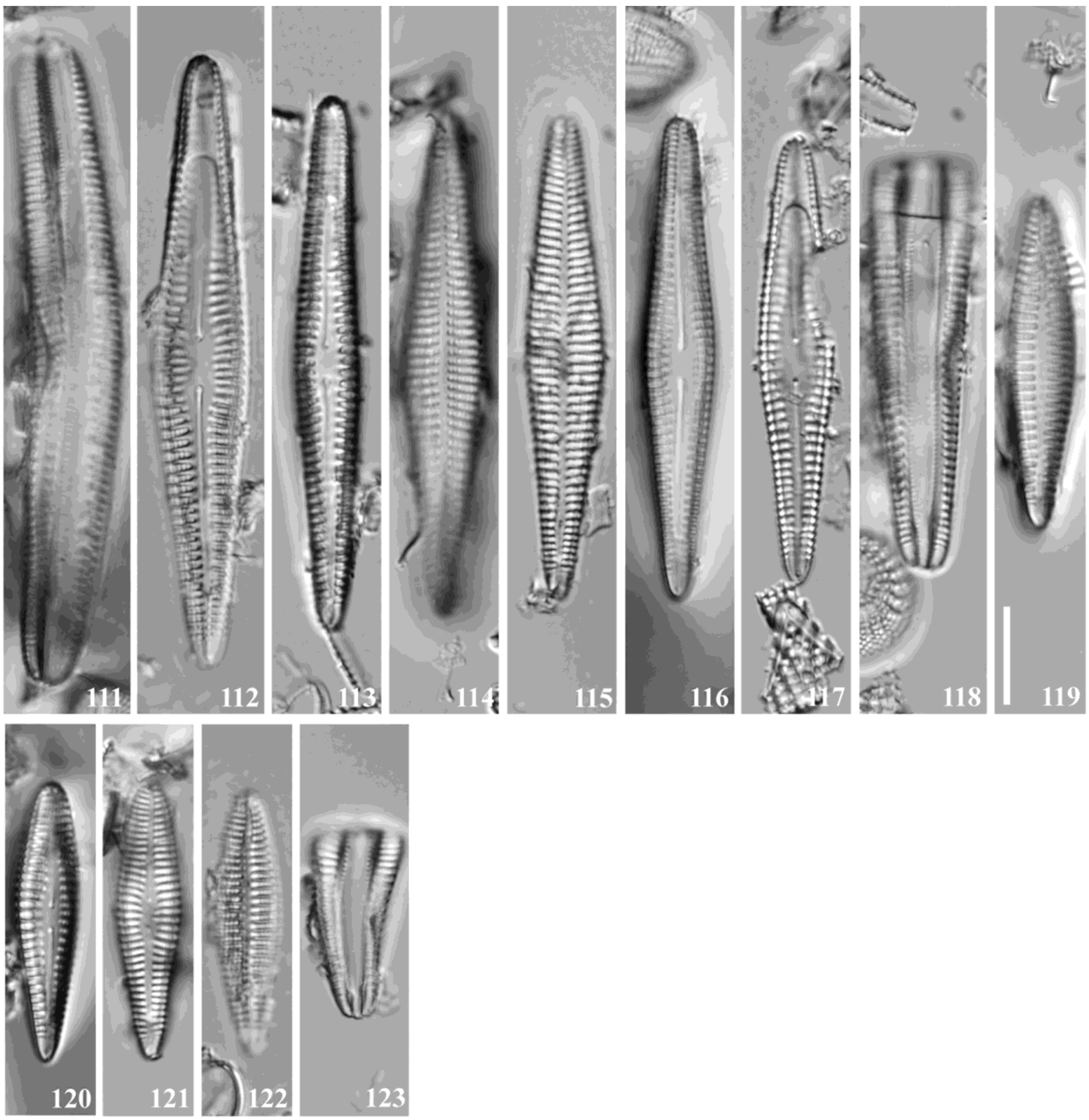
Isotype: Slide and material JPK 0357, University of Colorado, Museum of Natural History, Kociolek Collection, Boulder, Colorado, USA.

Type locality: Fossil deposit near Lower Bridge on Deschutes River, Oregon, United States of America. 44.359 N, 121.294 W, collected in July 1982 by M.C. Whiting.

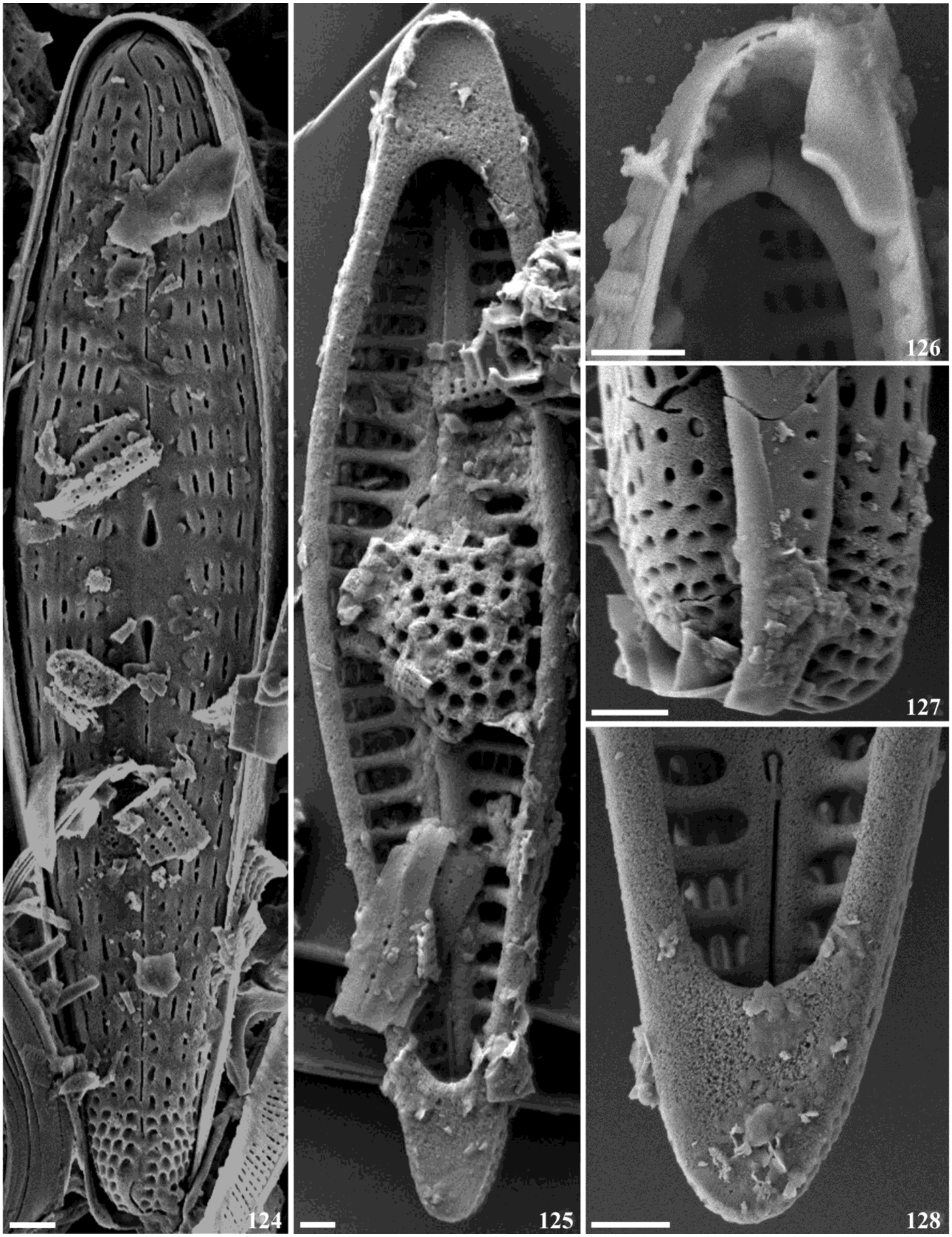
Taxonomic remarks: *Rhoicosphenia reimeri* can be distinguished by its shape from both *R. curvata* var. *subacuta* M. Schmidt and *R. curvata* var. *major* Cleve. *Rhoicosphenia reimeri* is more angular with a wide middle and drawn-out apices, whereas *R. curvata* var. *major* is more linear throughout the valve. *Rhoicosphenia reimeri* is also less flexed in girdle view (cf. Schmidt 1899, Pl. 213, Figs 15, 16). Furthermore, *R. reimeri* has distinct central and axial areas that differentiate it from *R. curvata* var. *subacuta*. *Rhoicosphenia reimeri* most closely resembles Schmidt's images of *R. curvata* var. *subacuta* from recent brackish material from the Caspian Sea (Schmidt 1899, Pl. 213, Figs 9, 10). It also has coarser, strongly radiate striae, in contrast to the finer, parallel striae of the Caspian Sea specimens. Schmidt's images of fossil specimens of *R. curvata* var. *subacuta* do not have the same shape as *R. reimeri*. Schmidt's image of a specimen from Mexico (Schmidt 1899, Pl. 213, Fig. 8) is linear-lanceolate, his specimen from Washington County is lanceolate, but it is not as inflated in the mid valve as *R. reimeri* (Schmidt 1899, Pl. 213, Fig. 19).

Distribution: Known only from type locality.

Etymology: Named in honor of Dr. Charles Reimer, a great American diatomist.



Figures 111–123: Type material of *Rhoicosphenia reimeri* from fossil deposit near Lower Bridge near Deschutes River, Oregon, USA. LM. Fig. 52. Holotype specimen. Figs 112–113, 116–117, 120. R-valves. Figs 114–115, 119, 121–122. D-valves. Figs 111, 118, 123. Girdle views showing slight flexure of frustules.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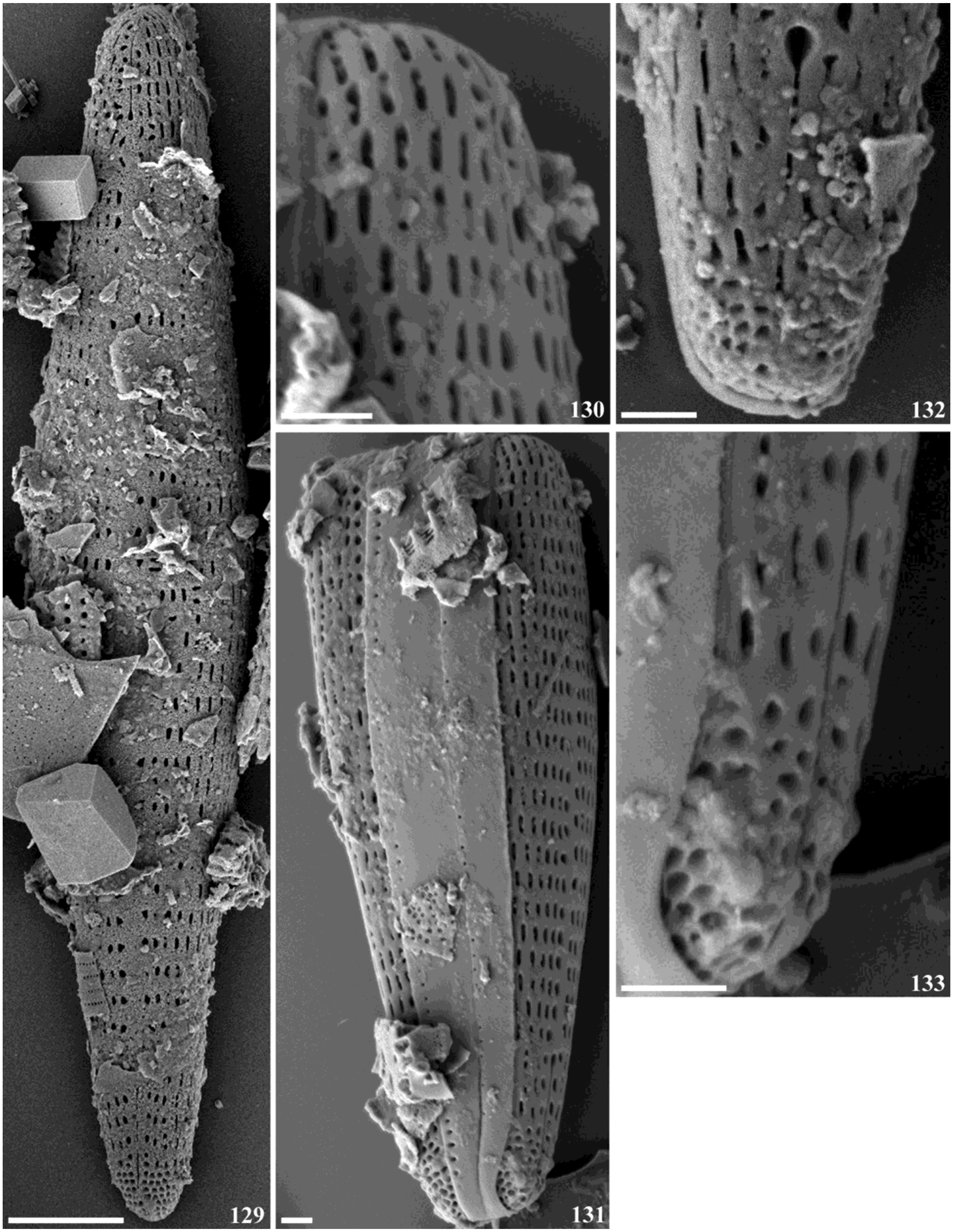
Figures 124–128: Type material of *Rhoicosphenia reimeri* from fossil deposit near Lower Bridge near Deschutes River, Oregon, USA. SEM. Fig. 124. Exterior of the R-valve has lineolate striae, large drop-shaped proximal raphe ends, distal raphe ends curved in the same direction, and condensed, rounded porelli arranged in and apical pore field. Fig. 125. Internally, troughs between the virgae are visible as well as lineolate exterior openings and prominent pseudosepta obscure helictoglossae at each pole. Fig. 126. Pseudoseptum visible through an aperture in the valvocopula. Fig. 127. Girdle view of foot pole shows distinct difference between areolae of valve and porelli of apical pore fields. Fig. 128. Internally, images of the D-valve show prominent pseudoseptum, recurved proximal raphe end at the foot pole, troughs between the virgae and lineolate external areolae. Scale bars = 1 µm.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Figures 129–133: Type material of *Rhoicosphenia reimeri* from fossil deposit near Lower Bridge near Deschutes River, Oregon, USA. SEM. Fig. 129. Externally, the D-valve has lineolate areolae and condensed, rounded porelli arranged in an apical pore field. Fig. 130. Raphe at the head pole is very short and the proximal end is slightly inflated. Figs 131, 133. The raphe branch at the foot pole has more prominently expanded, drop-shaped proximal ends and the raphe bisects the apical pore field and continues onto mantle. Fig. 132. In girdle view, the valve is weakly flexed, and the long, narrow lineolate areolae are evident as well as cingulum, each element with a single row of simple poroids. Scale bars = 5 um (Fig. 129), 1 μm (Figs 130–133).

***Rhoicosphenia patrickae* E.W. Thomas & Kociolek sp. nov.** (Figs 134–151)

Frustules clavate and strongly flexed in girdle view. Valves heteropolar, linear to narrow lanceolate with slightly protracted, rounded apices, 11–45 µm long, 4.0–4.5 µm wide. Frustules heterovalvate, one valve concave with elongated raphe (R-valve), one valve convex with shortened raphe branches (D-valve). R-valve raphes straight, proximal raphe ends expanded externally, recurved internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area narrow, central area small. Striae radiate at the center of the valve, becoming parallel to slightly convergent at the poles, 10–11 in 10 µm, composed of lineolate areolae, 40 per 10 µm. D-valve raphe 2–3 µm long at head pole and 2–4 µm long at foot pole, internal proximal ends recurved in same direction and distal ends terminate in helictoglossae internally. Reduced raphe branches reach end of pseudoseptum at head pole and beyond at foot pole. Striae parallel in center becoming radiate at poles, 10–11 in 10 µm. Both valves with pseudosepta at each apex, 3–5 µm long. Both valves with apical pore field at foot pole, porelli 5 per 1 µm. Girdle bands open.

As observed in the SEM, external R-valve shows rounded (Fig. 143) to lineolate (Figs 144, 145) areolae, most of those bordering the axial area are round (Figs 143, 145). Proximal raphe ends are rounded and dilated (Figs 143, 145). Apical pore field consists of obliquely arranged slit-like porelli, densely arranged and in general similar in appearance to the areolae (Fig. 144). In internal views of the R-valve the areolae can be seen in troughs between the virgae (Figs 146, 148) as well as pseudoseptum and broken valvocopula at foot pole (Figs 146–147). The valvocopulae are modified to fit over the pseudoseptum (Figs 146–147, 150). Proximal raphe ends are strongly recurved in the same direction in the interior of the R-valve (Figs 146, 148).

External D-valve has one row of rounded areolae near axial area transitioning to lineolate areolae that extend onto the mantle (Fig. 149). Apical pore field is small and comprised of obliquely oriented slit-like porelli, bisected by raphe branch with drop-shaped proximal end (Fig. 149). Raphe branch at head pole is very short, with slightly inflated drop-shaped proximal end and does not continuing onto mantle (Fig. 149). In internal views of the D-valve the areolae can be seen in troughs between the virgae showing the round to lineolate external areolae and shortened raphe branch at foot pole with recurved proximal end (Fig. 150). Valvocopula with simple internal poroids, extending to cover the pseudosepta (Fig. 150). Girdle view of frustule shows strong flexure of cell, as well as the cingulum, each girdle band with single row of simple poroids (Fig. 151).

Holotype: Circled specimen on slide ANSP GC 65211 made from material ANSP GCM 5689, Academy of Natural Sciences, Philadelphia, USA. Holotype specimen illustrated in Figure 64.

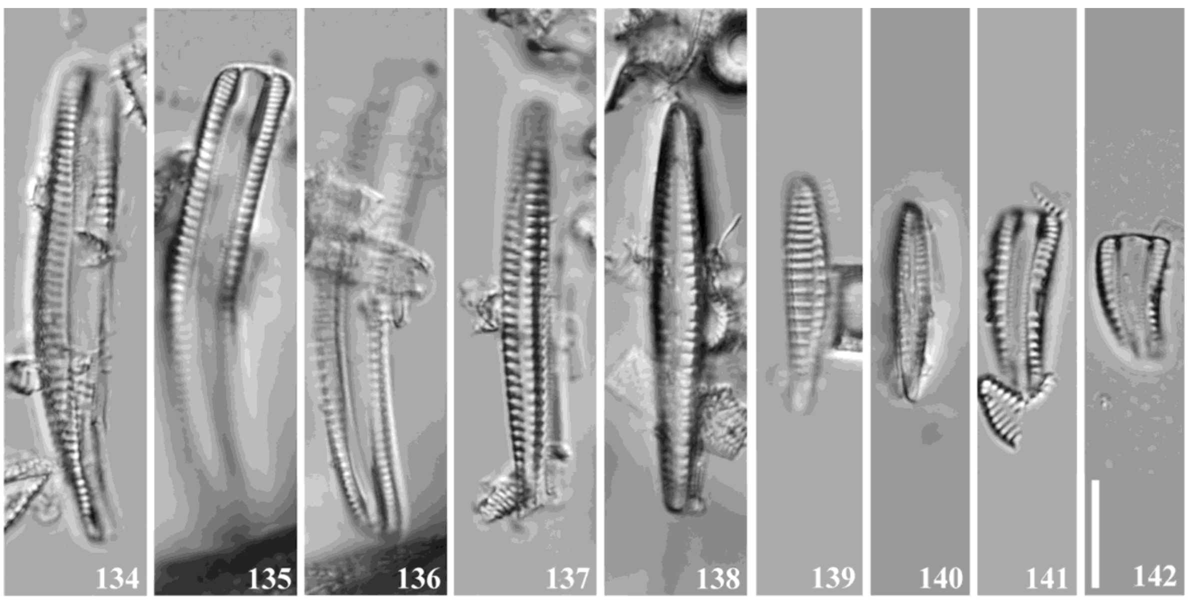
Isotype: Slide and material JPK 0357, University of Colorado, Museum of Natural History, Kociolek Collection, Boulder, Colorado, USA.

Type locality: Fossil deposit near Lower Bridge on Deschutes River, Oregon, United States of America. 44.359 N, 121.294 W, collected in July 1982 by M.C. Whiting.

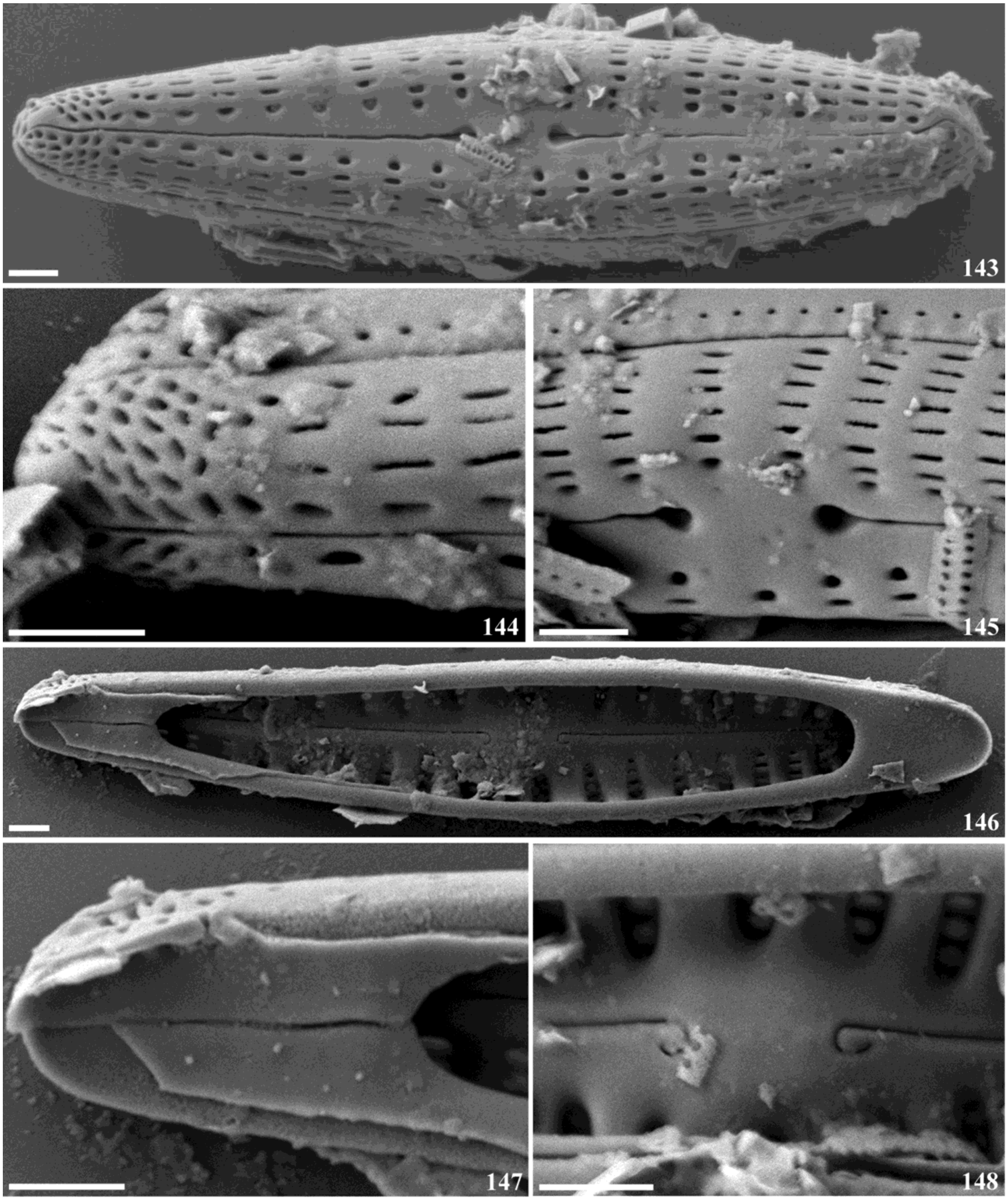
Taxonomic remarks: *Rhoicosphenia patrickae* is most similar in shape to *R. curvata* var. *gracilis* M. Schmidt. However, *R. patrickae* is less angular than *R. curvata* var. *gracilis* and from the center of the valve face to the apices the margins are convex, as opposed to concave as in *R. curvata* var. *gracilis*. Also, from Schmidt's image (Schmidt 1899, Pl. 213, Fig. 17) although the sizes overlap, the length to breadth ratio is different, *R. patrickae* is longer and narrower. Finally, *R. patrickae* has 10–11 striae in 10 μm and the striae are more radiate than Schmidt's *R. curvata* var. *gracilis*, which has 13 striae in 10 μm. The type localities of these taxa are in close proximity, *R. patrickae* from north-central Oregon, *R. curvata* var. *gracilis* from northern California ('Pitt River'). However, recent examinations of extant *Rhoicosphenia* in California illustrate that several distinct species can occur in close proximity with each other (personal observations).

Distribution: Known only from type locality.

Etymology: Named in honor of Dr. Ruth Patrick, a great American diatomist.



Figures 134–142: Type material of *Rhoicosphenia patrickae* from fossil deposit near Lower Bridge near Deschutes River, Oregon, USA. LM. Fig. 138. Holotype specimen. Figs 134–136, 141, 142. Girdle views showing strong flexure of frustules. Figs 138, 140. R-valves. Fig. 138. Pseudoseptum evident at head pole. Figs 137, 139. D-valves. Scale bar = 10 µm.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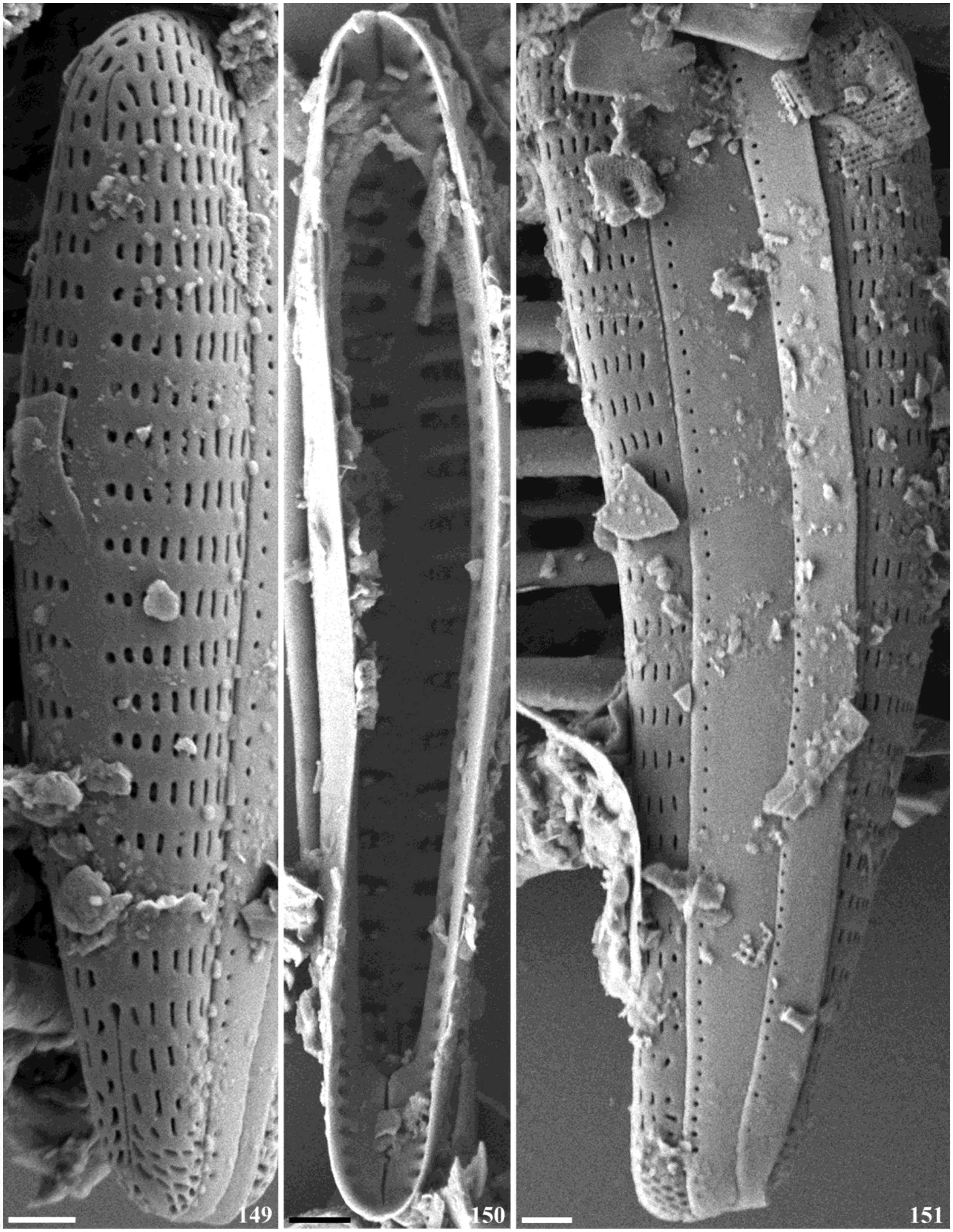
Figures 143–148: Type material of *Rhoicosphenia patrickae* from fossil deposit near Lower Bridge near Deschutes River, Oregon, USA. SEM. Figs 143, 145. External view of R-valve with rounded areolae, particularly beside the axial area, straight raphe and round and dilated proximal raphe ends. Fig. 144. Apical pore field consists of densely and obliquely arranged slit-like porelli, generally similar to the areolae. Fig. 146. Internal view of R-valve shows troughs between the virgae as well as pseudosepta and broken valvocopula at foot pole. Fig. 147. Foot pole with valvocopula that lacks an aperture. Figs 146, 148. Proximal raphe ends are strongly recurved in the same direction in the interior of the R-valve. Fig. 148. Rounded and lineolate external areolae opening are seen in troughs between the virgae. Scale bars = 1 µm.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Figures 149–151: Type material of *Rhoicosphenia patrickae* from fossil deposit near Lower Bridge near Deschutes River, Oregon, USA. SEM. Fig. 149. D-valve with one row of rounded areolae near axial area, transitioning to lineolate areolae that extend onto the mantle. Apical pore field comprised of obliquely oriented slit-like porelli, bisected by raphe branch with drop-shaped proximal end. Raphe branch at head pole is very short, with slightly inflated drop-shaped proximal end and does not continue onto mantle. Fig. 150. Internal D-valve with troughs between the virgae showing the round to lineolate external areolae and shortened raphe branch at foot pole with recurved proximal end. Valvocopula with simple internal poroids, extended to cover the pseudoseptum. Fig. 151. Girdle view of frustule shows strong flexure of cell, as well as the cingulum, each girdle band with single row of simple poroids. Scale bars = 1 μm.

A summary of the morphological traits of the new and similar taxa is given in Table 5.

Taxon	Habitat	Distribution	Length	Width	Striae (R)	Striae (D)
R. gandhii	Fossil	Gujarat, India	16–48	6–8.5	12–14	12–14
R. indica	Fossil	Gujarat, India	12.5–44	3–4	12–13	12–13
R. reimeri	Fossil	Oregon, USA	18–70	7–10	9–11	9–11
R. patrickae	Fossil	Oregon, USA	11–45	4–4.5	10–11	10–11
R. curvata var. *gracilis*	Fossil Freshwater	Pit River, USA	34	4.5	13	n/a
R. curvata var. *major*	Fossil/extant Freshwater	Pit River, USA	70	8	9	n/a
R. curvata var. *subacuta*	Fossil/extant Freshwater to marine	China, Europe, North America	34–76	6.5–9	8–15	9–15
R. fossilis	Fossil/marine	Kk. Knjäsha/ Russia	60	7	10	n/a
R. tenuis	Freshwater	Macedonia	15–60	3–5	12–16	13–17

Table 5: Taxon comparison and trait summary of fossil taxa. Information on habitat and morphology of four new species of *Rhoicosphenia* and taxa used for comparisons (Cleve 1895, M. Schmidt 1899, Cleve-Euler 1915, Cleve-Euler 1953, Levkov & Nakov 2008).

Discussion

Sample information and biogeographical notes

Fossil collections from India and western North America have yielded descriptions of four previously unknown fossil *Rhoicosphenia* species. Extant *Rhoicosphenia* taxa are rare (or absent) in India and were not included in a recent publication of common freshwater diatoms from India (Karthick et al. 2013). In the United States *Rhoicosphenia* commonly occurs in both marine and freshwater habitats and is found in many lists of species, from both floras and monitoring programs (Kociolek 2005, Bahls 2009, ANS et al. 2011–2016). Regarding fossil diversity in the Pacific Northwest of the United States, Schmidt illustrated three taxa, *R. curvata* var. *subacuta* M. Schmidt, *R. curvata* var. *major* Cleve, and *R. curvata* forma *minor* M. Schmidt, but none of these taxa are morphologically similar to the North American species described here, *R. reimeri* and *R. patrickae*. The taxa of the fossil ‘species swarm’ described from western North America by Schmidt (1899), in addition to the species presented in this paper, may indicate that more *Rhoicosphenia* species are awaiting discovery in the area. The Pacific Northwest has been shown to be rich in diatom diversity, including endemic species (Kociolek 2005, Bahls 2011, 2013), so the description of new fossil *Rhoicosphenia* species is in line with previous discoveries. Interestingly, both the two Indian species and the two Oregon species are found together in their respective type material. Each location has one larger, more coarsely ornamented species, *R. gandhii* and *R. reimeri*, and each has one smaller species, *R. indica* and *R. patrickae*. There is, however, no overlap in size or shape between the larger and smaller species within each sample, and independent size diminution series do not suggest that these represent different morphological life stages of a single species.

Neither of the samples have undergone any dating procedures, however, based on their diatom communities relative ages can be inferred. The Indian sample comes from a region abundant in fossils and because Gandhi did not record the age of the deposit, it is difficult to assess the deposit’s exact geological age. However, the range of diatom genera present (Table 6) suggest a Pliocene to Pleistocene age (Krebs et al. 1987, Benson et al. 2013). Similarly, no dating has been conducted on the sample from Oregon, but the region has many Pliocene to Pleistocene diatomites (Krebs et al. 1987, Benson et al. 2013) and the diatom community (Table 6) is similar to that of the Indian sample. Modern samples with *Rhoicosphenia* species have similar congeneric diatoms as the fossil samples in this study (cf. modern samples including *Rhoicosphenia* in the Academy of Natural Sciences of Drexel University, Philadelphia).

Mahi River, Gujarat, India	**Lower Bridge on Deschutes River, OR, USA**
Aulacoseira	*Aneumastus*
Caloneis	*Aulacoseira*
Campylodiscus	*Cocconeis*
Cocconeis	*Cosmioneis*
Cymbella	*Cymatopleura*
Encyonema	*Cymbella*
Epithemia	*Diploneis*
Fragilaria	*Encyonema*
Neidium	*Epithemia*
Pinnularia	*Fragilaria*
Rhopalodia	*Gomphoneis*
Sellaphora	*Nitzschia*
Stauroneis	*Pinnularia*
Staurosira	*Rhopalodia*
Staurosirella	*Sellaphora*
Stephanodiscus	*Stauroneis*
Surirella	*Staurosira*
	Stephanodiscus
	Surirella

Table 6: List of diatom genera found in samples with fossil taxa examined in this study.

With respect to the biogeography of the genus, *Rhoicosphenia* is most commonly reported from temperate zones of both the Northern and Southern Hemispheres. In the Northern Hemisphere, it has been reported extensively in Europe (Foged 1984a, Whitton et al. 2003, Hällfors 2004, Levkov et al. 2007) and the United States (Benson & Rushforth 1975, Lawson & Rushforth 1975, Clark & Rushforth 1977, Czarnecki & Blinn 1977, Grimes & Rushforth 1982) and Canada (Cumming et al. 1995, Reavie & Smol 1998, Pienitz et al. 2003), but also in the Canary Islands (Gil-Rodríguez et al. 2003), China (Hu & Wei 2006, Levkov et al. 2010), Mongolia (Østrup 1908), Pakistan (Leghari et al. 2005), and Russia (Skabichevskii 1976). In the Southern Hemisphere, there are reports from Chile (Rivera 1983), Uruguay (Metzeltin et al. 2005), Australia (Foged 1978), and New Zealand (Foged 1979, Harper et al. 2012). Reports of

Rhoicosphenia from the northern tropics include Columbia (Montoya-Moreno et al. 2013), Cuba (Foged 1984b), the Hawaiian Islands (Fungladda et al. 1983, Sherwood 2004), and Mexico's Yucatan Peninsula (Novelo et al. 2007), and from the southern tropics, Ghana (Foged 1966), India (Misra 1956), Burundi (Cocquyt 1998), New Caledonia (Moser 1999) and Papua New Guinea (Vyverman 1991). Finally, the fewest reports of any *Rhoicosphenia* species come from Polar Regions (Al-Handal & Wulff 2008). Overall, citations from temperate regions are richest in *Rhoicosphenia* diversity, including but not limited to, *R. abbreviata* and *R. curvata*, while only these two species have been reported from tropical regions, possibly due to the use of broad species concepts. To date, only three species, *R. adolfi* M. Schmidt (Schmidt 1899), *R. flexa* Giffen (Giffen 1970), and *R. genuflexa* (Kützing) Medlin (Medlin & Fryxell 1984b), and one variety, *R. marina* var. *intermedia* M. Schmidt (Schmidt 1899), have been described from the Southern Hemisphere, all of which are marine. Based on unpublished records from individual locations and literature citations, freshwater *Rhoicosphenia* diversity is greatest in northern temperate ecosystems. The discovery of new species in India is therefore unexpected.

Morphology

Members of *Rhoicosphenia* possess a suite of morphological characters that generated several hypotheses about the position of the genus in the diatom tree of life (Mann 1982a, Kociolek & Stoermer 1986). Some interesting features are its frustule asymmetry about both apical and transapical axes and valve flexure in girdle view. Another notable feature of *Rhoicosphenia* is the presence of shortened raphe branches on the D-valve, in contrast to the elongated raphe on the R-valve. Even though a great deal of morphological research has been done on *Rhoicosphenia* (Mann 1982a, Mann 1982b, Levkov et al. 2010), the septum-like structure on the valvocopula is often rarely mentioned. Despite the detailed assessment of the girdle bands, the only mention of a septum or septum-like structure is a reference to 'peculiar' pars interior and 'extensions of the pars interior across the pseudosepta' (Mann 1982a, Fig. 45). The septum-like structure was illustrated, but not referred to as a septum and there was no discussion of the ontogeny or possible homology of this feature with similar features in other diatom genera (Mann 1982a). In recent papers on *Rhoicosphenia* diversity, any modification of the valvocopula has also been rarely discussed (Levkov et al. 2007, Levkov & Nakov 2008) and only briefly mentioned in this passage: 'The valvocopula is modified to fit and interlock with the pseudosepta' (Levkov et al. 2010, pg. 146).

The septum-like structure or valvocopula modified to fit over the pseudoseptum (Levkov et al. 2010) in *Rhoicosphenia* is present on both valvocopulae at both poles and does not coalesce in the middle of the valve (Figs 147, 150), differentiating it from the interdigitating bars on the valvocopulae of *Diatomella balfouriana* Greville (Van de Vijver et al. 2012). The valvocopula of *Rhoicosphenia* fits tightly against the pseudoseptum and is only as long as the pseudoseptum (Figs 126, 147, 150). Unlike the septa of *Tabellaria* Ehrenberg, this valvocopula does not create a cavity within the frustule interior (or only a very small one between it and the similarly-sized pseudoseptum). Regarding interspecific variation of the valvocopula in *Rhoicosphenia*, two types are evident from images (Figs 126, 146, 147; Mann 1982a, Figs 12, 44–46, 50; Levkov et al. 2010, Figs 2d, 3d–f, 5e, 6c, 10f, 11c, f, g, 13d, 14e, 23d, 24b, c, g, h, 26d, 27b, e, f, 32e, h; Levkov & Nakov 2008, Figs 34, 35, 45, 49). *Rhoicosphenia gandhii* and *R. reimeri* have valvocopulae with an aperture (Fig. 126), while *R. indica* and *R. patrickae* lack an aperture (Figs 146, 147, 150). SEM images of *R. patrickae* show that the same type of valvocopula (without an aperture) is consistent present against both the R- and D-valves (Figs 147, 150). *Rhoicosphenia abbreviata* (Mann 1982a, Figs 12, 44–46; Levkov et al. 2010, Fig. 2d) also has an aperture in its valvocopulae. Other illustrations of an aperture on the valvocopula include *R. baltica* (Levkov et al. 2010, Fig. 5e), *R. lacustris* (Levkov et al. 2010, Fig. 23d), and *R. affinis* (Levkov et al. 2010, Fig. 26d), and *R. tenuis* (Levkov & Nakov 2008, Fig. 34). All these images show the valvocopula against the R-valve, but there are no images of the valvocopula on the D-valve. Some species presented by Levkov et al. (2010) lack SEM images of the feature, so this list cannot be exhaustive, but the fact that images are lacking shows the degree to which valvocopular morphology in *Rhoicosphenia* has been overlooked.

Since descriptions of *Rhoicosphenia* have ignored this feature, investigations of septa and septum-like structures in diatoms have not included *Rhoicosphenia* (Gotoh 1984, Cox 2012, Van de Vijver et al. 2012). However, our images and those in other papers suggest that septum-like structures are present in *Rhoicosphenia*, but they are not referred to as such (Mann 1982a, Levkov et al. 2007, 2010, Levkov & Nakov 2008). The most thorough, recent review of the criteria that must be met in order to be referred to as a septum, including a discussion on the septa and septum-like structures of many genera, can be found in Van de Vijver et al. (2012). Still, what is notable is the absence of any discussion in regards to the septum-like structures of *Rhoicosphenia* (Van de Vijver et al. 2012). *Diatomella* Greville and other raphid diatoms have modifications that arise at many points along the valvocopula, not from the longitudinal center (Van de Vijver et al. 2012, Fig. 16). Based on the valvocopula morphology in *Rhoicosphenia*, which resembles that of other raphid diatoms, we hypothesize that they may be produced differently from those in araphid taxa.

Two other genera, *Gomphonema* C.A. Agardh and *Gomphoneis* P.T. Cleve are also known to have structures referred to as septa (Kociolek & Stoermer 1988, Thomas et al. 2009), but not mentioned (Gotoh 1984, Van de Vijver et al. 2012) or thoroughly discussed (Cox 2012) in reviews of septa and septum-like structures. The raphid diatom genera are not closely related to araphids, and within the raphids, the genera with valvocopula modified in some way are also not closely related to each other, with the exception of *Gomphonema* and *Gomphoneis* (Sims et al. 2006, Ruck & Theriot 2011). Although *Gomphonema* was shown to be sister to *Rhoicosphenia* (Kociolek & Stoermer 1986), molecular data has thus far been unable to conclusively provide support for the position of *Rhoicosphenia* in the diatom tree of life (Nakov et al. 2014). Therefore, the valvocopular modifications of *Rhoicosphenia* may not be homologous with the similar feature in Gomphonemoid diatoms and they are likely the result of convergent evolutionary processes.

In conclusion, if the definition of homology is ‘having a common evolutionary origin’ (Patterson 1988), it would be proper to assess whether or not the diatoms with these structures are closely related. Two hypotheses for presence of a septum are possible; the first being that the septum is a pleisiomorphic trait that is secondarily lost in many taxa, or that it has evolved independently in several lineages that are not closely related, a convergent feature. A septum is found several araphid genera including *Tabellaria* Ehrenberg, *Tetracyclus* Ralfs, *Oxyneis* Round (Tabellariaceae Kützing), and *Licmophora* C. Agardh of the Licmophoraceae Kützing, *Rhabdonema* Kützing of the Rhabdonemataceae Round & Crawford, and *Striatella* C. Agardh, *Microtabella* Round, *Pseudostriatella* S. Sato, D.G. Mann & Medlin, and *Grammatophora* Ehrenberg of the Striatellaceae Kützing (Van de Vijver et al. 2012). In the raphid diatoms discussed in a recent review, the genera *Gomphoseptatum* Medlin, *Denticula* Kützing, *Epithemia* Kützing, and *Diatomella* possess septum-like structures (Van de Vijver et al. 2012). *Gomphonema*, *Gomphoneis*, and *Rhoicosphenia* are other raphid diatoms with septum-like structures. Septa, septum-like structures, and other valvocopular modifications are all ways to describe a feature of the valvocopulae, although based on their placement in the diatom tree, these features seem to have been independently derived several times, an example of convergent evolution. Patterson (1988) summarizes this parallelism as the transition of a structure, i.e. the valvocopula that can be modified in similar ways, but in unrelated taxa. Similar characters are therefore derived by no more than convergent processes (Patterson 1988). Convergent evolutionary processes seem to offer the most likely scenario for the possession of septa, septum-like structures (Gotoh 1984), scalariform valvocopulae (Van de Vijver et al. 2012), and other valvocopular modifications in unrelated taxa.

Acknowledgements

The authors would like to thank Dr. Jennifer Ress and two anonymous reviewers for constructive critique of this manuscript.

Note on published manuscripts

The geographical focus of the preceding papers were the states California and Oregon on the west coast of the US. This was done because intensive sampling of streams in California was completed for the Southern California Bight (SCB) project by SCCWRP, and the Surface Water Ambient Monitoring Program (SWAMP) by the State of California. These sampling events allowed me to have access to hundreds of samples containing *Rhoicosphenia* that I could then go and re-sample for molecular analyses. In addition to these previously mentioned samples, I was also able to examine *Rhoicosphenia* from across the US by visiting the Academy of Natural Sciences of Drexel University (ANS) in Philadelphia, PA. Based on observations made at ANS, I provide descriptions and light and scanning electron micrographs of several new morphologies observed from across the US.

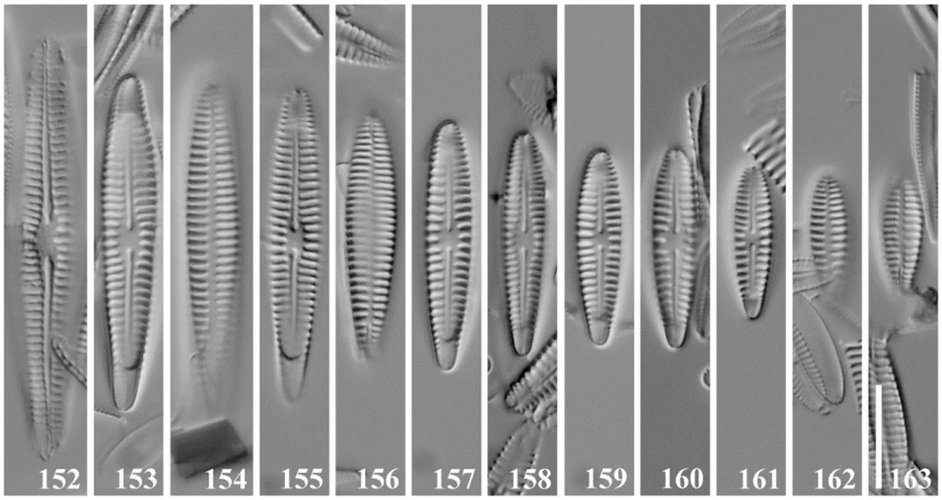
Descriptions of new morphospecies from the US

Rhoicosphenia sp. 1 (Figs 152–163)

Frustules clavate and slightly flexed in girdle view. Valves heteropolar in valve view, linear-clavate with rounded apices, 12–51 µm long, 4–6 µm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). *R-valve* raphe filiform, proximal raphe ends slightly expanded, recurved internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area narrow, central area small and ovate. Striae parallel to slightly radiate in center of the valve and radiate towards apices, 10–14 in 10 µm, composed of round areolae towards axial area, lineolate areolae towards mantle, 40 in 10 µm. *D-valve* raphe 1–3 µm long at head pole, not extending beyond pseudoseptum, and 1–3 µm long at foot pole, external proximal ends straight, internal proximal ends recurved in same direction and distal ends not inflated externally, terminate in helictoglossae internally. Striae parallel throughout, 10–14 in 10 µm, composed of round areolae towards axial area, lineolate areolae towards mantle. Both valves with pseudosepta at each apex, 2–4 µm long. Both valves with apical pore field at foot pole, porelli 4 per 1 µm. Girdle bands open.

Taxonomic remarks: *Rhoicosphenia sp. 1* is distinguished from other *Rhoicosphenia* taxa by its shape, size, and rounded apices. Compared to *R. abbreviata*, as documented in Levkov et al. (2010), *R. sp. 1* is narrower and has a higher striae density. Of the *Rhoicosphenia* species described from the US (Thomas & Kociolek 2015), it is most similar to *R. californica*, however, *R. sp. 1* is more clavate than lanceolate, has higher striae density, and is narrower in comparably sized specimens.

Imaged population locality: Washington, USA, 47.56843 N, 122.18178 W, ANS 111324a.



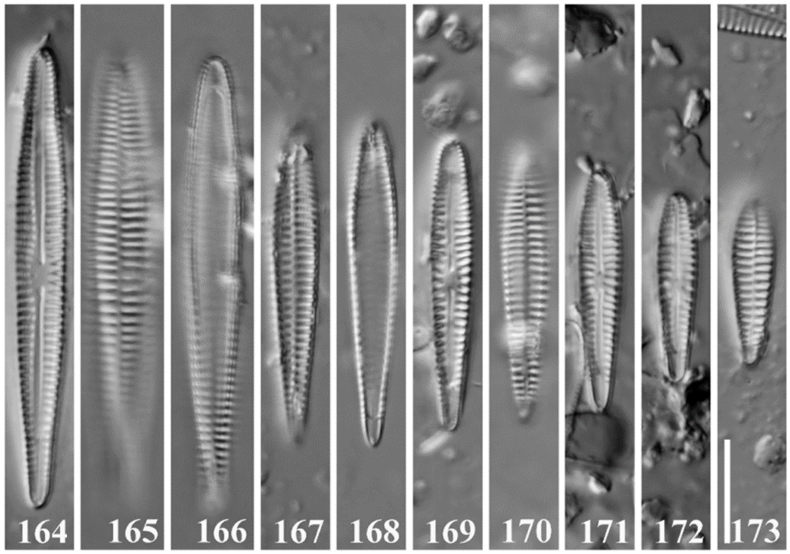
Figures 152–163: *Rhoicosphenia sp. 1* size diminution series. Scale bar = 10 µm.

Rhoicosphenia sp. 2 (Figs 164–173)

Frustules clavate and flexed in girdle view. Valves heteropolar in valve view, narrowly linear-lanceolate with narrow, attenuated apices, 13–45 μm long, 3–4.5 μm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). *R-valve* raphe filiform, proximal raphe ends expanded, recurved internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area narrow, central area small and linear. Striae slightly radiate to parallel in center of the valve and radiate at apices, 10–14 in 10 µm, composed of round areolae towards axial area, lineolate areolae towards mantle, 30 in 10 µm. *D-valve* raphe 1–3 µm long at head pole, not extending beyond pseudoseptum, and 1–3 µm long at foot pole, external proximal ends straight, internal proximal ends recurved in same direction and distal ends not inflated externally, terminate in helictoglossae internally. Striae slight radiate to parallel in center, radiate at apices, 10–14 in 10 µm, composed of round areolae towards axial area, lineolate areolae towards mantle. Both valves with pseudosepta at each apex, 1–3 µm long. Both valves with apical pore field at foot pole, porelli 3 per 1 µm. Girdle bands open.

Taxonomic remarks: *Rhoicosphenia sp. 2* is distinguished from *R. sp. 1* due to its linear-lanceolate, rather than linear-clavate valve shape and its narrower valves with attenuated apices. It can also be distinguished from *R. californica* due its higher striae density, as well as its symmetry, as the widest point of *R. sp. 2* is above mid-valve, while *R. californica* is widest at mid-valve. *Rhoicosphenia sp. 2* is distinguished from *R. abbreviata* due to shape and the fact that *R. sp. 2* is narrower, and *R. sp. 2* has higher striae density.

Imaged population locality: White Earth River, North Dakota, USA, 48.36710 N, -102.77721 W, ANS 114728b.



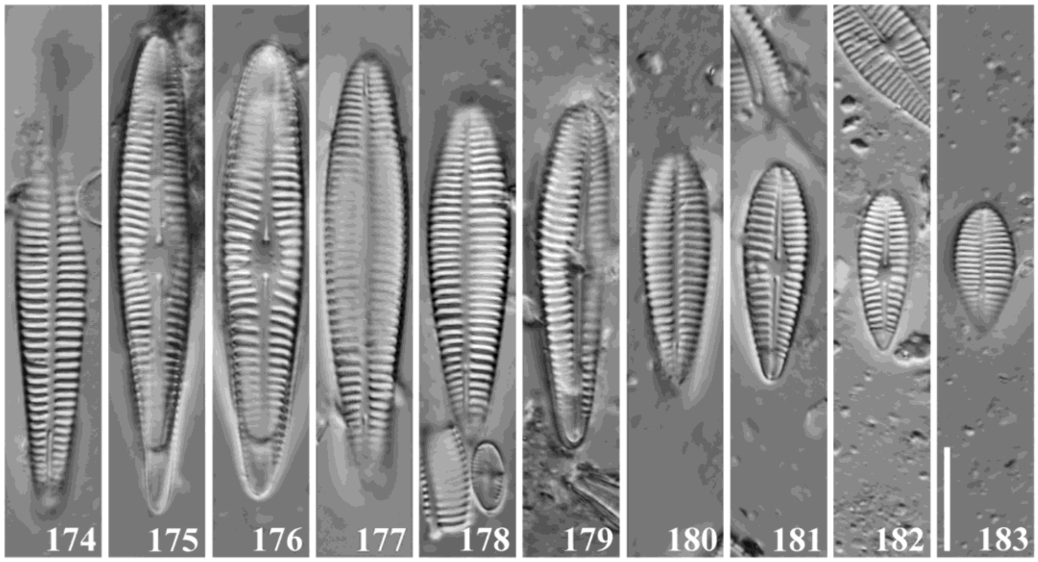
Figures 164–173: *Rhoicosphenia sp. 2* size diminution series. Scale bar = 10 µm.

Rhoicosphenia sp. 3 (Figs 174–183)

Frustules clavate and evenly flexed in girdle view. Valves heteropolar in valve view, widely lanceolate-clavate with rounded apices, 10–53 µm long, 5–8 µm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). *R-valve* raphe filiform, proximal raphe ends dilated, recurved internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area linear and tapering towards central area, central area large and ovate. Striae radiate in center of the valve and radiate at apices, 11–13 in 10 µm, composed of lineolate areolae, 40 in 10 µm. *D-valve* raphe 2–3 µm long at head pole, not extending beyond pseudoseptum, and 4–6 µm long at foot pole, external proximal ends dilated, internal proximal ends recurved in same direction and distal ends not inflated externally, terminate in helictoglossae internally. Striae linear throughout, very slightly radiate at apices, 11–13 in 10 µm, composed of lineolate areolae. Both valves with pseudosepta at each apex, 3–5 µm long. Both valves with apical pore field at foot pole, porelli 5 per 1 µm. Girdle bands open.

Taxonomic remarks: *Rhoicosphenia sp. 3* is most similar to *Rhoicosphenia sp. 5*, but differs in having coarser striae and rounded head and foot poles. It also differs from *R. abbreviata* because specimens of *R. sp. 3* of the same length are wider.

Imaged population locality: Maumee River, Ohio, USA



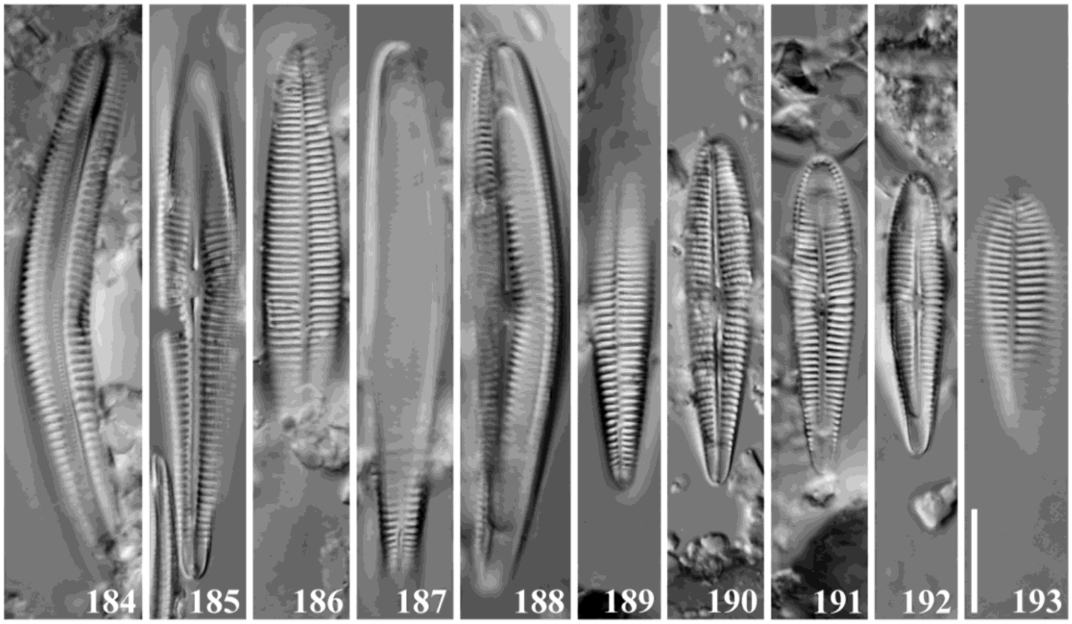
Figures 174–183: *Rhoicosphenia sp. 3* size diminution series. Scale bar = 10 µm.

Rhoicosphenia sp. 4 (Figs 184–193)

Frustules clavate and highly flexed in girdle view, with distinct kink at mid-valve. Valves heteropolar in valve view, widely lanceolate-clavate with rounded head pole and acute foot pole, 27–57 µm long, 6–8 µm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). *R-valve* raphe straight, proximal raphe ends slightly dilated, recurved internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area narrow, central area slightly panduriform in large specimens, small and ovate in small specimens. Striae radiate throughout, 13–16 in 10 µm, composed of lineolate areolae, 50 in 10 µm. *D-valve* raphe 1–2 µm long at head pole, not extending beyond pseudoseptum, and 2–5 µm long at foot pole, external proximal ends straight, internal proximal ends recurved in same direction and distal ends not inflated externally, terminate in helictoglossae internally. Striae parallel in center, radiate at apices, 13–14 in 10 µm, composed of lineolate areolae. Both valves with pseudosepta at each apex, 3–6 µm long. Both valves with apical pore field at foot pole, porelli 4 per 1 µm. Girdle bands open.

Taxonomic remarks: The shape, high striae density, and extreme flexure in girdle view of *R. sp. 4* distinguishes it from all other *Rhoicosphenia* taxa.

Imaged population locality: Rio Arriba, New Mexico, USA, 36.0739 N, -106.1111 W, ANS 101199b.



Figures 184–193: *Rhoicosphenia sp. 4* size diminution series. Scale bar = 10 µm.

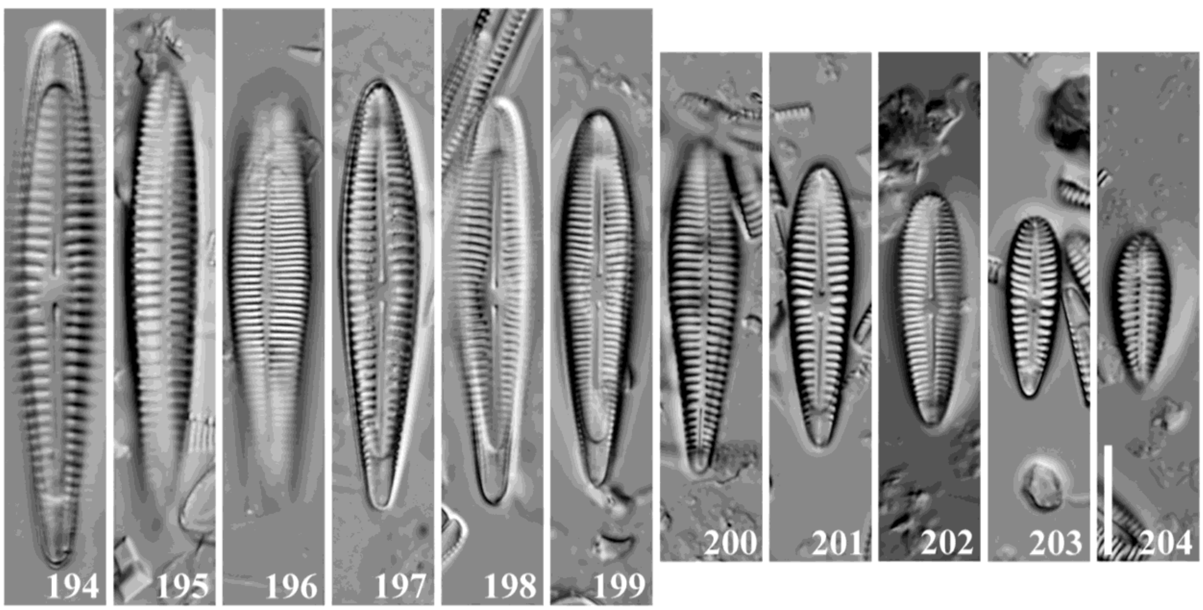
Rhoicosphenia sp. 5 (Figs 194–204)

Frustules clavate and curved throughout in girdle view. Valves heteropolar in valve view, narrowly ovate with head pole bluntly pointed in large specimens and rounded apices in smallest specimens and narrowly acute foot pole, 15–50 µm long, 5–7 µm wide. Frustules heterovalvate, one valve concave with long raphe branches (R-valve), one valve convex with shortened raphe branches (D-valve). *R-valve* raphe straight, proximal raphe ends inflated externally, recurved internally in same direction, distal raphe ends curved in same direction externally ending in helictoglossae internally. Axial area narrow and linear, central area ovate and slightly inflated. Striae parallel to slightly radiate in center of the valve, parallel towards apices and radiate at apices, 11–16 in 10 µm at center of valve, 14–18 in 10 µm at apices, composed of lineolate areolae, 40 in 10 µm. *D-valve* raphe 2–3 µm long at head pole, not extending beyond pseudoseptum, and 3–4 µm long at foot pole, external proximal ends not expanded, internal proximal ends recurved in same direction and distal ends not inflated externally, terminate in helictoglossae internally. Striae parallel in center, radiate at apices, 11–16 in 10 µm at center of valve, 14–16 in 10 µm at apices, composed of lineolate areolae. Both valves with pseudosepta at each apex, 3–4 µm long. Both valves with apical pore field at foot pole, porelli 4 per 1 µm. Girdle bands open.

Taxonomic remarks: *Rhoicosphenia sp. 5* is similar to several *Rhoicosphenia* species, but many features can be used to distinguish it based on morphology. *Rhoicosphenia sp. 5* is similar to various interpretations of *R. abbreviata* (Patrick and Reimer 1966, Pl. 20, Figs 1–5; Krammer & Lange-Bertalot 1986, Fig. 91, images 20–28; Levkov et al. 2010, Figs 1a–p), but the two taxa differ mainly in valve shape as *R. sp. 5* has obovate valves, more linear margins and has a denser striae than *R. abbreviata* (11–16 in 10 µm versus 9–12 in 10 µm as reported in Levkov et al.

2010). *Rhoicosphenia sp. 5* is also similar to *R. curvata* var. *subacuta* in size and striae density, but the shape of the valves is different as *R. sp. 5* is more linear and the foot pole is not as narrow as *R. curvata* var. *subacuta* (in Schmidt 1899, Pl. 213, Figs 11–14). *R. macedonica* Levkov & Krstic (in Levkov et al. 2010, Figs 9a–t) is similar in shape, but *R. sp. 5* is narrower, up to 7 µm versus 8.5 µm wide, and has less dense striae, 11–16 in 10 µm versus 18–22 in 10 µm for *R. macedonica*.

Imaged population locality: Aliso Creek, California, USA. 33.516368 N, 117.740624 W.



Figures 194–204: *Rhoicosphenia sp. 5* size diminution series. Scale bar = 10 µm.

Concluding remarks

Based on the morphological evidence presented in this chapter, it has been shown that the species diversity of *Rhoicosphenia* is far greater than previous investigations had demonstrated. The new species presented were only observed in streams, ignoring all other habitats such as ponds, lakes, and wet rock faces. While we do not know whether or not any of the taxa discussed here would be found in non-stream habitats, the taxa presented here are not meant to be an exhaustive list of *Rhoicosphenia* in the US. That is to say, further investigations may reveal more undescribed diversity in the US.

CHAPTER III

POSITION OF *RHOICOSPHENIA* IN DIATOM PHYLOGENY

This chapter has been published in PLOS ONE and the full citation is:

Thomas, E.W., Stepanek, J.G. & Kociolek, J.P. (2016) Historical and Current Perspectives on the Systematics of the 'Enigmatic' Diatom Genus *Rhoicosphenia* (Bacillariophyta), with Single and Multi-Molecular Marker and Morphological Analyses and Discussion on the Monophyly of 'Monoraphid' Diatoms. *PLOS ONE* 11(4): e0152767.

http://dx.doi.org/10.1371/journal.pone.0152797

Abstract

This study seeks to determine the phylogenetic position of the diatom genus *Rhoicosphenia*. Currently, four hypotheses based on the morphology of the siliceous valve and its various ultrastructural components, sexual reproduction, and chloroplasts have been proposed. Two previous morphological studies have tentatively placed *Rhoicosphenia* near members of the Achnanthidiaceae and Gomphonemataceae, and no molecular studies have been completed. The position of *Rhoicosphenia* as sister to 'monoraphid' diatoms is problematic due to the apparent non-monophyly of that group, so hypotheses of 'monoraphid' monophyly are also tested. Using an analysis of morphological and cytological features, as well as sequences from three genes, SSU, LSU, and *rbc*L, recovered from several freshwater *Rhoicosphenia* populations that have similar morphology to *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot, we have analyzed the phylogenetic position of *Rhoicosphenia* in the context of raphid diatoms. Further, we have used topology testing to determine the statistical likelihoods of these relationships. The hypothesis that *Rhoicosphenia* is a member of the Achnanthidiaceae cannot be rejected, while the hypothesis that it is a member of the Gomphonemataceae can be rejected. In our analyses, members of the Achnanthidiaceae are basal to *Rhoicosphenia*, and *Rhoicosphenia* is basal to the Cymbellales, or a basal member of the Cymbellales, which includes the Gomphonemataceae. Hypothesis testing rejects the monophyly of ‘monoraphid’ diatoms.

Keywords: *Rhoicosphenia*, phylogeny, SSU, LSU, *rbc*L, morphology, ‘monoraphid’, *Achnanthidium*, *Cocconeis*, Cymbellales, *Gomphonema*

Introduction

Of the tremendous diversity found in the diatoms, one monophyletic group is the pennate diatoms (Theriot et al. 2010). Pennate diatoms may possess a raphe, a pair of slits through the glass cell wall that allows diatoms with this structure to micro-position themselves when in contact with a substratum. Some diatoms have a raphe system on both valves of their bipartite frustules (called biraphid diatoms), while others have a raphe system on one valve only (termed monoraphid diatoms). The systematic position of the raphid diatom genus *Rhoicosphenia* Grunow (Grunow 1860) has been the subject of considerable interest and debate from its inception as a distinct genus and for the subsequent 150 years. *Rhoicosphenia* was erected based on *Gomphonema curvata* Kützing (Kützing 1833) as the generitype and was differentiated from *Gomphonema* Ehrenberg (Ehrenberg 1831) by having valves flexed about the transapical axis and shortened raphe branches on the convex valve. *Rhoicosphenia* was originally placed in the ‘monoraphid’ family Achnantheae (Grunow 1860), which also included *Achnanthes* Bory (1822–1831) *sensu lato*, (at the time both *Achnanthes sensu stricto* and *Achnanthidium* Kützing (Kützing 1844) were considered part of this genus) and *Cocconeis* Ehrenberg (Ehrenberg 1835).

This systematic placement close to *Achnanthidium* within the 'monoraphid' diatoms has been followed by some workers (Peragallo 1897, Cleve-Euler 1953, Hustedt 1959, Patrick & Reimer 1966, Chen & Zhu 1983).

After the description of *Rhoicosphenia*, Van Heurck (1896) articulated what was the first alternate hypothesis regarding its phylogenetic position and placed it within the biraphid Tribe Gomphonemeae, citing similarities in chloroplast morphology between *Rhoicosphenia* and *Gomphonema*. Several diatomists of the 19th and 20th centuries agreed with this position (De Toni 1891–4, Simonsen 1979). After Van Heurck, Mereschkowsky (1902) noted that based on chloroplast structure, *Rhoicosphenia* was part of the raphid group Pyrenophoreae, which are united by a single chloroplast with a central pyrenoid. Within the Pyrenophoreae, Mereschkowsky also suggested the closest relative of *Rhoicosphenia* to be *Gomphonema* (Mereschkowsky 1902), with both genera being in the Tribe Gomphonemeae. Mereschkowsky's Pyrenophoreae was part of the larger group, the Monoplacatae, along with another group of note, the Heteroideae (Mereschkowsky 1902). Genera included in the Pyrenophoreae and considered in our paper were *Anomoeoneis* Pfitzer (1871), *Cymbella* Agardh (1830), *Encyonema* Kützing (1833), and *Placoneis* Mereschkowsky (1903), while the Heteroideae included the genera *Cocconeis* and *Microneis* Cleve (1895) (now *Achnanthidium*). Cleve (1895) provided a less concrete placement of *Rhoicosphenia* due to his interpretation of 'monoraphid' diatoms as not a 'natural' group, i.e. polyphyletic, while Schütt (1896) hypothesized it to be a 'Bindeglied zwischen' (translated as 'link between') *Gomphonema* and *Achnanthes*, and Schütt's view was illustrated in Peragallo (1897).

Rhoicosphenia and *Gomphonema*, are currently placed in the Cymbellales Mann (Round et al. 1990), while Achnanthidium is placed in the Achnanthales Silva (1962). Round et al.

(1990) proposed the following genera to be in the Cymbellales: *Anomoeoneis* (Anomoeoneidaceae), *Placoneis*, *Cymbella*, *Encyonema* (Cymbellaceae), *Gomphonema*, *Didymosphenia* M. Schmidt (1899), *Gomphoneis* Cleve (1894), and *Reimeria* Kociolek & Stoermer (1987) (Gomphonemataceae), and *Rhoicosphenia* (Rhoicospheniaceae Chen & Zhu (1983)). *Cymbopleura* Krammer (1999), *Geissleria* Lange-Bertalot & Metzeltin (1996), and *Encyonopsis* Krammer (1997) were erected and remained in the Cymbellales and molecular analyses have supported their placement (Kulikovskiy et al. 2014, Nakov et al. 2014), while several other genera are included in the order (Round et al. 1990), but have not been formally analyzed with either morphological or molecular data. 'Gomphonemoid' diatoms include four genera in Kützing's (1844) Gomphonemataceae, but morphological and molecular analyses revealed that *Gomphonema* and *Gomphoneis* should be in the family, while *Didymosphenia* and *Reimeria* are more closely related to members of the Cymbellaceae (Kociolek & Stoermer 1987, Nakov et al. 2014, Kociolek & Stoermer 1988). Thus, for this paper, we consider only *Gomphonema* and *Gomphoneis* to be 'gomphonemoid' diatoms. When we refer to the Cymbellales we are doing so in the expanded sense of Round et al. (1990), with inclusion of *Cymbopleura*, *Geissleria* and *Encyonopsis*, but excluding *Rhoicosphenia*, as we are testing its phylogenetic position.

Genera in the Achnanthales per Round et al. (1990) include *Achnanthes* (Achnanthaceae), *Cocconeis* (Cocconeidaceae), and *Achnanthidium* (Achnanthidiaceae). These are often referred to as 'monoraphid' diatoms, due to the presence of a raphe system on one valve only, and over the past two decades several genera including *Karayevia* Round & Bukhtiyarova ex (Round 1998), *Lemnicola* Round & Basson (Round 1997), *Planothidium* Round & Bukhtiyarova (1996), *Platessa* Lange-Bertalot in (Krammer & Lange-Bertalot 2004), *Psammothidium* Bukhtiyarova & Round (1996), and *Rossithidium* Round & Bukhtiyarova ex (Round 1998) have been proposed and include many species assigned previously to *Achnanthidium* and other genera in this group. Molecular data have been generated for some of these taxa, and the position of *Achnanthes sensu stricto* has been shown (Ruck & Theriot 2011, Kociolek et al. 2013, Stepanek & Kociolek 2014) distinct from other 'monoraphid' genera, such as *Achnanthidium*, *Cocconeis*, and *Lemnicola*. Based on the distant phylogenetic position of *Achnanthes sensu stricto*, we will here take a narrower view of 'monoraphid' diatoms and include the genera *Achnanthidium*, *Cocconeis*, *Lemnicola*, *Planothidium*, and *Psammothidium*, but exclude *Achnanthes*. The distant phylogenetic position of *Achnanthes* relative to the other aforementioned monoraphid genera was proposed by Mereschkowsky (1902) and has been supported by molecular phylogenies (Sims et al. 2006, Bruder & Medlin 2008a). Mereschkowsky (1902) placed *Achnanthidium* (then *Microneis*) and *Cocconeis* into the Heteroideae, which excluded *Achnanthes*, so we will test whether *Rhoicosphenia* is part of a monophyletic group with taxa in the Heteroideae.

In the 1980's, there was substantial interest in the phylogenetic position of *Rhoicosphenia* (Mann 1982a, Mann 1982b, Mann 1984, Medlin & Fryxell 1984a, Medlin & Fryxell 1984b, Kociolek & Stoermer 1986). Mann (1982a) asserted four hypotheses for the systematic position of *Rhoicosphenia*, which are paraphrased as follows (Figure 1);

1) a) *Rhoicosphenia* is an intermediate form between *Achnanthes* and *Gomphonema*, or,

   b) The common ancestor of 'monoraphid' and 'gomphonemoid' genera,
2) *Rhoicosphenia* is a 'monoraphid' diatom,
3) *Rhoicosphenia* is related to *Gomphonema*, and
4) *Rhoicosphenia* is unrelated to 'monoraphid' and gomphonemoid diatoms.

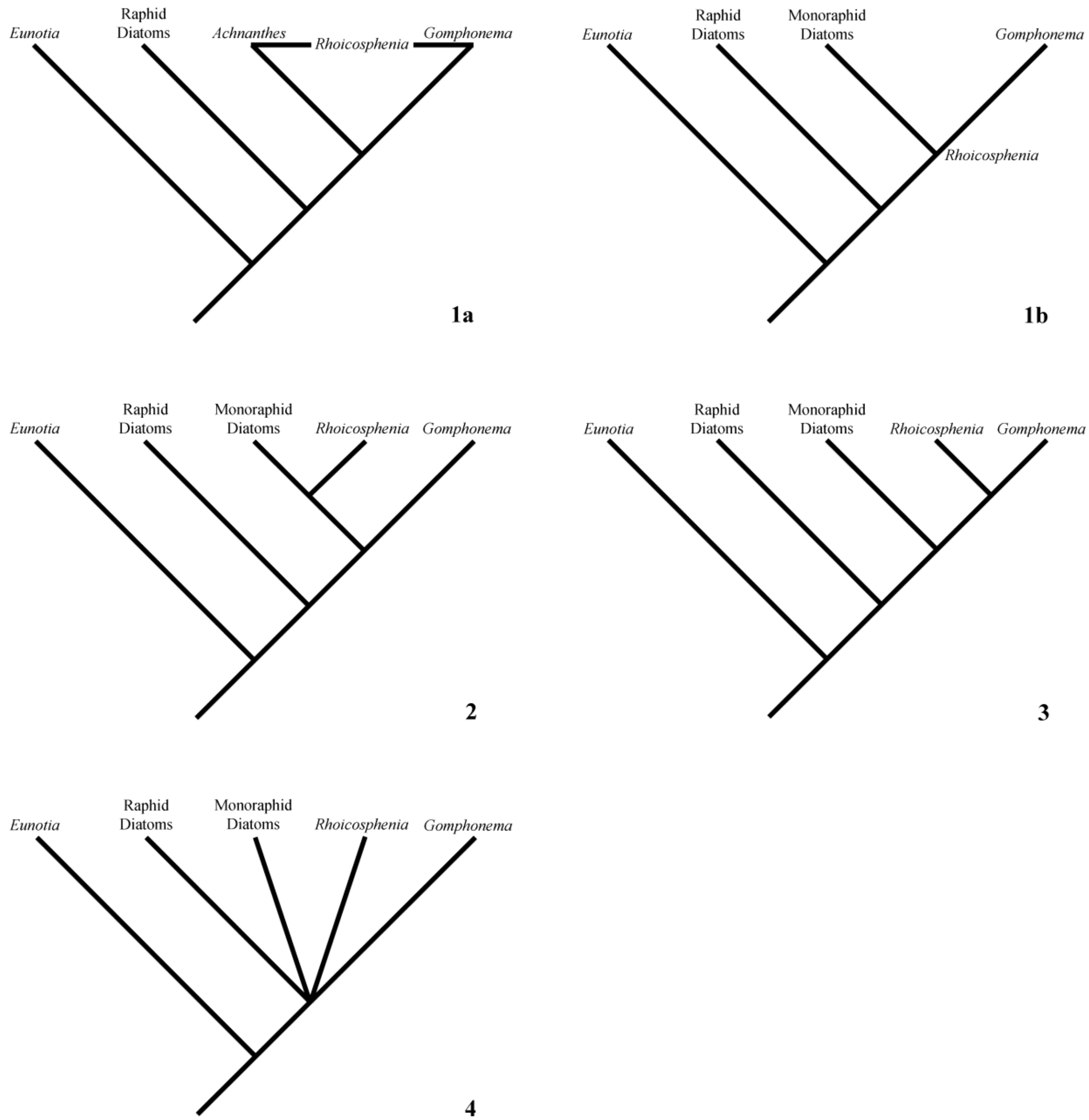


Figure 1: Summary of historical hypotheses.

Hypothesis 1 has two parts; (a) *Rhoicosphenia* is an intermediate form between *Achnanthes* and *Gomphonema*, and (b) is the common ancestor of both ‘monoraphid’ and gomphonemoid groups. Hypothesis 1a was proposed by Schütt (1896) with *Rhoicosphenia* being the link between *Gomphonema* and *Achnanthes*, but we are unable to test the topology with our statistical methods and will therefore not statistically address the hypothesis in this paper. Hypothesis 1b is not testable with hypothesis testing techniques, since *Rhoicosphenia* would not occupy a position as a terminal taxon, but rather be placed at a node of divergence between ‘monoraphid’ and gomphonemoid diatoms. However, the hypothesis will be tested broadly in the context of the position of *Rhoicosphenia* compared to other genera. Hypothesis 2 (Mann 1982a) follows Grunow and Hustedt, with *Rhoicosphenia* being more closely related to ‘monoraphid’ diatoms. Hypothesis 3 (Mann 1982a) follows Van Heurck and Mereschkowsky and states that *Rhoicosphenia* is sister to *Gomphonema*. Finally, hypothesis 4 (Mann 1982a) most closely resembles Cleve’s hypothesis that the phylogenetic affinity of *Rhoicosphenia* to ‘monoraphid’ diatoms is due to polyphyletic origins of the ‘monoraphid’ condition, but also does not lend itself to hypothesis testing because we cannot place *Rhoicosphenia* in an unknown position in the tree. In studying the morphology of *Rhoicosphenia* valves in detail, some of Mann’s (Mann 1982a) conclusions were that the valve symmetry of *Rhoicosphenia* is similar to *Gomphonema* and *Cymbella*, *Rhoicosphenia* valves are not similar to *Achnanthes* or *Cocconeis*, the chloroplasts of *Rhoicosphenia* are more similar to *Achnanthidium* than *Achnanthes* (and cites Mereschkowsky’s (1902) chloroplast work), and *Rhoicosphenia* is unlike *Gomphonema* due to areolar occlusions differences (Mann 1982a). Subsequently, Mann notes differences in sexual reproduction between the isogamous *Rhoicosphenia* and the physiological anisogamy of *Gomphonema* and *Cymbella* (Mann 1982b). The conclusions of Mann’s final paper support the 4th hypothesis, that

Rhoicosphenia ‘clearly’ is not allied with ‘monoraphid’ diatoms, but belongs in an ‘isolated position’ near the gompho-cymbelloid diatoms within the Naviculales and offers an emended description of the family Rhoicospheniaceae (Mann 1984).

Soon after Mann’s papers, a cladistic analysis of *Cocconeis, Mastogloia* Thwaites in (Smith 1856), *Achnanthes sensu lato, Gomphonema*, and *Rhoicosphenia* was produced (Kociolek & Stoermer 1986). Using eleven morphological characters to test historical hypotheses similar to those in Mann (1982a), the analysis showed that *Rhoicosphenia* is more closely related to *Gomphonema*, with *Achnanthes sensu lato* as sister and *Cocconeis* more distantly related (Kociolek & Stoermer 1986). In that analysis, *Rhoicosphenia* did not occupy an undetermined position, but was sister to *Gomphonema* and only closely allied with one of the other ‘monoraphid’ genera, *Achnanthes sensu lato*. A more recent cladistic analysis using morphology that included *Rhoicosphenia* employed more characters (n=35) and taxa (n=49). This analysis placed *Rhoicosphenia* in an unresolved polytomy of raphid diatoms (Cox & Williams 2006). These subsequent results do not support Grunow’s hypothesis of relationship, based on his decision to place his ‘newly’ erected genus in the Achnantheae, and also rejects the hypothesis that *Rhoicosphenia* is sister to *Gomphonema*. The results showed that some members of Cymbellales *sensu* Mann in (Round et al. 1990), (*Cymbella*, *Encyonema*, *Gomphonema*, and *Reimeria*) are a natural group, but *Anomoeoneis*, *Placoneis* and *Rhoicosphenia* were not allied with that group (Cox & Williams 2006). Also, the ‘monoraphid’ diatoms in that study, *Achnanthidium* and *Cocconeis*, formed a natural group, but *Rhoicosphenia* was excluded from that clade (Cox & Williams 2006). In terms of the four hypotheses forwarded by Mann, the study by (Cox & Williams 2006) supports hypothesis 4, that *Rhoicosphenia* occupies an ‘enigmatic’ position in the raphid diatom phylogeny (Mann 1982a, Mann 1984). Cox (2006) discussed several morphological characters of *Achnanthes sensu stricto* and suggested it belongs in the Mastogloiales Mann in (Round et al. 1990), rather than Achnanthales, again casting doubt on the monophyly of 'monoraphid' diatoms, supporting proposals made at the turn of the 20th century (Mereschkowsky 1902, Cleve 1895). *Rhoicosphenia* is also interesting because two of its potential phylogenetic positions, 'monoraphid' or *Gomphonema* (Cymbellales), are consistently returned as sister taxa in molecular analyses (Theriot et al. 2010, Ruck & Theriot 2011, Kociolek et al. 2013, Stepanek & Kociolek 2014, Bruder & Medlin 2008a, Kooistra et al. 2003, Medlin & Kaczmarska 2004, Sorhannus 2004), but many of these analyses are focused on other questions and have not discussed this relationship (Round et al. 1990, Nakov et al. 2014, Jones et al. 2005, Kermarrec et al. 2011, Mann & Stickle 1995).

Two additional hypotheses are added that are not strictly related to *Rhoicosphenia*, but more broadly to 'monoraphid' diatoms. The first, H_5, addresses the issue of whether or not all 'monoraphid' diatoms are monophyletic. Several molecular and one morphological (Cox & Williams 2006) have suggested that this is not the case, as *Achnanthes sensu stricto* is not part of a monophyletic group with the other 'monoraphid' diatoms, such as *Achnanthidium* and *Cocconeis*, and in fact is quite distantly related to them. The second, H_6, tests the hypothesis, forwarded by Cox (2006), that *Achnanthes sensu stricto* is closely related to the genus *Mastogloia*.

The major goal of this project is to use single and multi-marker molecular analyses, as well as analysis of morphological data to determine the systematic position of *Rhoicosphenia* in the diatom tree of life within the context of previous taxonomic hypotheses.

Materials and Methods

Molecular Analyses

Taxon collections: Three *Rhoicosphenia* populations were isolated from freshwater streams into monoculture via micro-pipette serial dilution from collections made in California, Colorado and Oregon, USA, and were grown in freshwater WC medium (Guillard & Lorenzen 1972). After isolation, the cultures were maintained at a temperature of approximately 25C, with a 12:12 light dark cycle at an irradiance of 50 µmol cm^{-2} s^{-1}. The other 4 sets of sequences were obtained via a Chelex extraction from colonies found in live samples. Colonies were chosen to ensure that DNA was obtain from one genetic clonal line. Table 1 contains information on sampling locations of sequenced specimens. Samples in California were collected with a Scientific Collecting Permit from the California Department of Fish and Wildlife, issued to Evan W. Thomas. The Oregon Department of Fish and Wildlife and Colorado Department of Natural Resources did not require permits for microalgal collections. All collections were made from state, county, and city parks, or from waterways accessible from public roads and no field sites had endangered or protected species.

Name	**State**	**Site Name**	**Latitude**	**Longitude**	**Type**
Rhoicosphenia 1 EWT	CO	Golden Ponds	40.1674	-105.1417	Culture
Rhoicosphenia 2 EWT	CO	Gaynor Lake	40.1168	-105.1056	Culture
Rhoicosphenia 3 EWT	CA	Mission Creek	34.4126	-119.6913	Chelex
Rhoicosphenia 4 EWT	CA	Penasquitos Creek	32.9439	-117.08	Chelex
Rhoicosphenia 37 EWT	OR	Hood River	45.7101	-121.5071	Chelex
Rhoicosphenia 80 EWT	OR	Willamette River	44.6380	-123.1602	Chelex
Rhoicosphenia 94 EWT	OR	McKenzie River	44.0558	-122.8281	Culture

Table 1: Sampling location information *Rhoicosphenia* populations sequenced including taxon Name and ID, State, Site Name, Latitude, Longitude, and type of extraction.

Seven *Rhoicosphenia* populations were sequenced for this analysis with 7 isolates yielding partial 18S small subunit rDNA (SSU) sequences, 6 sequences from the D1–D2 region of the 28S large subunit rDNA (LSU), and 4 sequences from the chloroplast encoded large subunit of RUBISCO (*rbc*L). Only three populations yielded sequences for all 3 markers. The list of populations studied, including taxon name, ID, sampling location information, and GenBank accession numbers is presented in Table 2.

Name	**ID**	**SSU**	**LSU**	***rbc*L**
Rhoicosphenia cf. *abbreviata*	1 EWT	KU965564	KU965571	KU965577
Rhoicosphenia cf. *abbreviata*	2 EWT	KU965565	KU965572	KU965578
Rhoicosphenia stoermeri	3 EWT	KU965566	KU965573	KU965579
Rhoicosphenia cf. *abbreviata*	4 EWT	KU965567	KU965574	n/a
Rhoicosphenia cf. *abbreviata*	37 EWT	KU965568	KU965575	n/a
Rhoicosphenia cf. *abbreviata*	80 EWT	KU965569	n/a	KU965580
Rhoicosphenia cf. *abbreviata*	94 EWT	KU965570	KU965576	n/a

Table 2: GenBank accession numbers for sequenced *Rhoicosphenia* populations.

Additionally, GenBank was used to obtain an additional 140 sequences for SSU, 80 sequences for LSU, and 100 sequences for *rbc*L and a list of these taxa are included in Table 3.

Full name with Authority	**Culture ID**	**SSU**	**LSU**	***rbc*L**
Achnanthes brevipes	CCMP100	AY485476		
Achnanthes cf. *longipes*	CCMP101	AY485500.1		
Achnanthes coarctata	FD185	HQ912594.1		HQ912458.1
Achnanthes sp.	CCAP1001/1	AY485496		
Achnanthes sp. 1	ECT3684	KC309476		KC309548.1
Achnanthes sp. 1	ECT3883	KC309474.1		KC309546.1
Achnanthes sp. 1	ECT3911	KC309475.1		KC309547.1
Achnanthes sp. 1	SanNic1	KC309473.1		
Achnanthidium minutissimum	AT-196Gel02	AM502032	AM710588	AM710499
Achnanthidium minutissimum	RK6	KF417666.1		
Achnanthidium minutissimum	TCC746	KF959663.1		
Adlafia brockmannii	AT-111Gel10	AM502020	AM710576	AM710487

Amphora libyca	AT-117.10	AM501959	AM710513	AM710425
Amphora pediculus	AT-117.11	AM501960	AM710514	AM710426
Anomoeoneis fogedii	FD399	KJ011610	KJ011555	KJ011793
Anomoeoneis sculpta	CH239	KJ011611	KJ011556	KJ011794
Anomoeoneis sphaerophora	FD160	KJ011612	KJ011557	KJ011795
Bacillaria paxillifer	FD468	HQ912627		HQ912491
Berkeleya rutilans	ECT3616	HQ912637		
Caloneis budensis	AT-220.06	AM502003	AM710559	AM710470
Caloneis lauta	AT-160Gel04	AM502039	AM710595	AM710506
Campylodiscus clypeus	L951	HQ912412		
Campylodiscus sp.	3613.8	HQ912413		
Climaconeis riddleae	ECT3724	HQ912644		
Cocconeis pediculus	AT-212.07	AM502010	AM710566	AM710477
Cocconeis placentula	AT-212Gel11	AM502013	AM710569	AM710480
Cocconeis stauroneiformis	S0230	AB430614.1	AB430654.1	AB430694.1
Craticula cuspidata	AT-200.05	AM501998	AM710554	AM710465
Craticula importuna	AT-70Gel14a	AM501978	AM710533	AM710444
Craticula molestiformis	AT-36.klein	AM501989	AM710532	AM710455
Cylindrotheca closterium	CCMP1855	HQ912645		
Cymatopleura elliptica	L1333	HQ912659		HQ912523
Cymbella affinis	AT-204Gel02	AM502009	AM710565	AM710476
Cymbella aspera	AT-210Gel07	AM502016	AM710572	AM710483
Cymbella cistula	CH019	KJ011618	KJ011562	KJ011801
Cymbella helvetica	B457	KJ011621	KJ011565	KJ011804
Cymbella janischii	CH062	KJ011622	KJ011566	KJ011805
Cymbella lanceolata	AT-194Gel07	AM502026	AM710582	AM710493
Cymbella mexicana	CH031	KJ011624	KJ011568	KJ011807
Cymbella proxima	AT-210Gel13	AM502017	AM710573	AM710484
Cymbella stuxbergii	B382	KJ011628	KJ011572	KJ011811
Cymbella tumida	1vii097A	KJ011629	KJ011573	KJ011812
Cymbopleura naviculiformis	AT-177.04	AM501997	AM710553	AM710464
***Cymbopleura* sp.**	TN-2014 B37	KJ011633	KJ011577	KJ011816
Didymosphenia dentata	B547	KJ011635	KJ011579	KJ011818
Didymosphenia geminata	CH058	KJ011636	KJ011580	KJ011819
Didymosphenia siberica	B40	KJ011637	KJ011581	KJ011820
Diploneis subovalis	FD282	HQ912597		

Diprora haenaensis	8296-Dipr001	KC954571		
Encyonema caespitosum	AT-214Gel03	AM502035	AM710591	AM710502
Encyonema macedonicum	CH011	KJ011638		
Encyonema minutum	22vi092A	KJ011640	KJ011582	KJ011823
Encyonema muelleri	16vi091B	KJ011642	KJ011584	KJ011825
Encyonema norvegicum	FD342	KJ011643		
Encyonema triangulum	2vii091	KJ011645	KJ011586	KJ011828
***Encyonopsis* sp.**	CH021	KJ011646	KJ011587	KJ011829
Entomoneis ornata	14A	HQ912411		
Entomoneis sp.	CS782	HQ912631		
Eolimna minima	AT-70Gel18	AM501962	AM710516	AM710427
Epithemia argus	CH211	HQ912408		
Epithemia turgida	CH154	HQ912410		HQ912396
Eunotia formica	AT-111Gel09	AM502040	AM710517	AM710428
Eunotia implicata	AT-219.07	AM502001	AM710557	AM710468
***Eunotia* sp.**	AT-73Gel02	AM501963	AM710518	AM710429
Fallacia monoculata	FD254	HQ912596		
Fallacia pygmaea	FD294	HQ912605		
Geissleria decussis	FD50	KJ011647	KJ011588	KJ011830
Gomphoneis minuta	CH053	KJ011648	KJ011589	KJ011831
Gomphonema acuminatum	AT-219Gel10	AM502019	AM710575	AM710486
Gomphonema affine	AT-196Gel03	AM502033	AM710589	AM710500
Gomphonema brebissonii	FD373	KJ011653	KJ011593	KJ011836
Gomphonema carolinense	FD285	KJ011654	KJ011594	KJ011837
Gomphonema* cf. *angustatum	AT-109Gel08b	AM502005	AM710561	AM710472
Gomphonema* cf. *parvulum	AT-161.15	AM501995	AM710551	AM710462
Gomphonema dichotomum	FD288	KJ011655	KJ011595	KJ011838
Gomphonema gracile	FD65	KJ011656	KJ011596	KJ011839
Gomphonema intricatum	FD383	KJ011658	KJ011598	KJ011841
Gomphonema micropus	AT-117.09	AM501964	AM710519	AM710430
Gomphonema parvulum	FD240	KJ011659	KJ011599	KJ011842
Gomphonema productum	AT-160Gel27	AM501993	AM710549	AM710460
***Gomphonema* sp.**	CH024	KJ011662	KJ011602	KJ011845
***Gomphonema* sp.**	CH026	KJ011663	KJ011603	KJ011846
***Gomphonema* sp.**	CH027	KJ011664	KJ011604	KJ011847
***Gomphonema* sp. 1LB**	B559	KJ011660	KJ011600	KJ011843
Gomphonema subclavatum* var. *commutatum	FD98	KJ011665	KJ011605	KJ011848

Gomphonema truncatum	AT-195Gel09	AM501956	AM710598	AM710509
Gyrosigma acuminatum	FD317	HQ912598		
Halamphora normannii	AT-105Gel05	AM501958	AM710512	AM710424
Hantzschia amphioxys var. *major*	A4	HQ912404		
Hippodonta capitata	AT-124.24	AM501966	AM710521	AM710432
Lemnicola hungarica	FD456	HQ912626.1		HQ912490.1
Mastogloia sp.	29X07-6B	HQ912632		HQ912496
Mayamaea atomus* var. *atomus	AT-115Gel07	AM501968	AM710523	AM710434
Mayamaea atomus* var. *permitis	AT-101Gel04	AM501969	AM710524	AM710435
Navicula gregaria	AT-117Gel05	AM501974	AM710529	AM710440
Navicula radiosa	AT-114Gel06	AM502034	AM710527	AM710501
Navicula reinhardtii	AT-124.15	AM501976	AM710531	AM710442
Navicula tripunctata	AT-202.01	AM502028	AM710584	AM710495
Neidium affine	FD127	HQ912583		
Neidium bisulcatum var. *subampliatum*	FD417	HQ912591		
Neidium productum	FD116	HQ912582		
Nitzschia amphibia	FDCC L602	AJ867277		
Nitzschia communis	FDCC L408	AJ867278	AF417661	
Nitzschia filiformis	FD267	HQ912589		
Pinnularia mesolepta	AT-160Gel30	AM501994	AM710550	AM710461
Pinnularia microstauron	AT-105Gel08	AM501981	AM710536	AM710447
Placoneis abiskoensis	FD363	KJ011667	KJ011607	KJ011850
Placoneis clementis	FD419	KJ011668		
Placoneis elginensis	AT-160Gel18	AM501953	AM710548	AM710459
Placoneis elginensis	FD212	KJ011669	KJ011608	KJ011852
Placoneis elginensis	FD416	HQ912607		
Placoneis hambergii	AT-160Gel09	AM502030	AM710586	AM710497
***Placoneis* sp.**	AT-220.09	AM502014	AM710570	AM710481
Planothidium frequentissimum	RK12	KF417663.1		
Planothidium lanceolatum	L1249	AJ535189.1		
Planothidium sp.	05DB5	KF417664.1		
Prestauroneis integra	AT-177.13	AM502025	AM710581	AM710492
Psammothidium papilio	FLB10	KM116121.1		

Psammothidium papilio	FLB11	KM116122.1		
Reimeria sinuata	TCC719	JN790290.1		
Reimeria sinuata	TCC721	JN790292.1		
Reimeria sinuata	TCC735	JN790291.1		
Rhopalodia contorta	L1299	HQ912406		HQ912392
Rhopalodia gibba	CH155	HQ912407		HQ912393
Rhopalodia sp.	9vi08.1F.2	HQ912405		
Rossia sp.	CH2	AJ535144		
Rossia sp.	E3333	EF151968		
Scoliopleura peisonis	FD13	HQ912609		
Sellaphora cf. *minima*	BM42	EF151966		
Sellaphora cf. *seminulum*	TM37	EF151967		
Sellaphora pupula	AUS1	EF151982		EF143312
Stauroneis anceps	AT-160Gel11	AM502008	AM710564	AM710475
Stauroneis gracilior	AT-117Gel17	AM501988	AM710543	AM710454
Stauroneis phoenicenteron	AT-117.04	AM501987	AM710542	AM710453
Stenopterobia curvula	L541	HQ912416		
Surirella angusta	SANG1	AJ867028		
Surirella minuta	FD320	HQ912658		HQ912522
Surirella splendida	19C	HQ912415		HQ912401
Tryblionella apiculata	FD465	HQ912600		HQ912464

Table 3: Additional (non-*Rhoicosphenia*) GenBank sequences used in analyses. Accession numbers appear below the molecular marker used in the analyses. Bold taxon names were used in three marker concatenated phylogeny.

DNA extraction amplification and sequencing: A Chelex 100® method (Richlen & Barber 2005) was used to extract DNA from monocultures and was modified to a volume of 20 µL Chelex for colonies of *Rhoicosphenia*. The molecular markers chosen, include the conserved (SSU) and variable (LSU, *rbc*L), which have been shown to provide order (Theriot et al. 2010, Ruck & Theriot 2011, Bruder & Medlin 2008a, Bruder & Medlin 2008b) and species (Alverson et al. 2007, Hamsher et al. 2010, Souffreau et al. 2011) level resolution. Further, due to the widespread use of these markers in diatom phylogenetics (Theriot et al. 2010, Nakov et al. 2014, Ruck & Theriot 2011, Kociolek et al. 2014, Stepanek & Kociolek 2014, Bruder & Medlin 2008a, 53, Bruder & Medlin 2008b, Alverson et al. 2007, Souffreau et al. 2011), it allowed for the broadest taxon sampling of non-*Rhoicosphenia* GenBank sequences from the raphid diatoms. Primers used in amplification and sequencing of these markers are listed in Table 4.

Primer Name	**Primer Sequence (5′ to 3′)**	**Reference**
SSU Primers		
SSU1[a]	AAC CTG GTT GAT CCT GCC AGT	(Medlin et al. 1988)
SSU850+	GGG ACA GTT GGG GGT ATT CGT A	(Ruck & Theriot 2011)
SSU870-	TAC GAA TAC CCC CAA CTG TCC C	(Ruck & Theriot 2011)
ITS1DR[a]	CCT TGT TAC GAC TTC ACC TTC C	(Edgar & Theriot 2004)
LSU Primers		
D1R[a]	ACC CGC TGA ATT TAA GCA TA	(Scholin et al. 1994)
D2C[b]	CCT TGG TCC GTG TTT CAA GA	(Scholin et al. 1994)
***rbc*L Primers**		
*rbc*L66+[a]	TTA AGG AGA AAT AAA TGT CTC AAT CTG	(Alverson et al. 2007)
*rbc*L404+	GCT TTA CGT TTA GAA GAT ATG	(Ruck & Theriot 2011)
*rbc*L1255-	TTG GTG CAT TTG ACC ACA GT	(Alverson et al. 2007)
dp7-[a]	AAA SHD CCT TGT GTW AGT YTC	(Daugbjerg & Andersen 1997)

Table 4: Primers used in amplification and sequencing of SSU, LSU, and *rbc*L. [a] Forward PCR amplification primer, [b] Reverse PCR amplification primer.

Using GE Healthcare illustra Ready-To-Go™ PCR beads (GE Healthcare Biosciences, Pittsburgh, Pennsylvania) following the manufacturer's instructions, all markers were amplified by polymerase chain reaction (PCR). PCR was performed in an Eppendorf Mastercycler® using the program: 94 C for 3:30, 36 cycles of 94 C for 50 seconds, 52 C for 50 seconds, 72 C for 80 seconds, with a final extension at 72 C for 15 minutes. After amplification, the PCR products were purified with ExoSAP-IT (Affymetrix, Santa Clara, California) using the manufacturers protocol. Purified PCR products were sequenced at Functional Biosciences, Inc. (Madison, Wisconsin) and Geneious ver. 5.6 (Drummond et al. 2012) was used to assemble and edit sequences. Sequences for the seven *Rhoicosphenia* taxa included in this analysis are deposited in GenBank and accession numbers for SSU, LSU, and *rbc*L sequences are listed in Table 1.

Sequence alignment and phylogenetic analysis: A muscle alignment algorithm (Edgar 2004) in Geneious was used for all alignments. The three molecular markers were aligned separately prior to concatenation in the two and three-molecular marker alignments. The ends were trimmed from each of the alignments to minimize missing characters. A variable 63 base pair region of SSU, corresponding to region 579–641 in the initial alignment, was removed due to the ambiguity in the alignment, creating a final trimmed length of 1566 sites. The final trimmed length of LSU was 604 base pairs and *rbc*L had a final trimmed length of 799 base pairs. The three-marker concatenated alignment for 81 taxa was 2969 sites. The SSU alignment included 140 non-*Rhoicosphenia* taxa with representatives from all available raphid diatom orders *sensu* (Round et al. 1990). The LSU and *rbc*L alignments included less taxa, but attempted to maintain coverage of raphid diatom groups based on available sequences. The number of taxa included in alignments are as follows: SSU – 147; LSU – 86; *rbc*L – 104; SSU + LSU – 85; SSU + *rbc*L – 97; LSU + *rbc*L – 81; and SSU + LSU + *rbc*L – 81. To understand the position of *Rhoicosphenia* in the diatom tree of life, both maximum likelihood (ML) and Bayesian analyses were performed all single, two-gene, and three-molecular marker alignments. The alignments can be accessed at figshare (https://figshare.com) and their DOI is 10.6084/m9.figshare.3115522 (S1 File: SSU + LSU + *rbc*L; S2 File: SSU + LSU; S3 File: SSU + *rbc*L; S4 File: LSU + *rbc*L; S5 File: SSU; S6 File: LSU; S7 File: *rbc*L). All seven alignments were analyzed using the general time reversible (GTR) model with a gamma distribution (Γ) and a proportion of invariable sites (I) (Theriot et al. 2010, Stepanek & Kociolek 2014). SeaView version 4.3.4 (Gouy et al. 2010) was used to perform maximum likelihood (ML) analysis with PhyML version 3.0 (Guindon et al. 2010) using the GTR+ Γ+I model with four rates classes and 500 bootstrap replicates to estimated branch support. MrBayes version 3.2.1 (Ronquist et al. 2012) was used to perform Bayesian analyses. Analyses were run using the default settings and a GTR+Γ+I model with four rate classes. The single and two-molecular marker alignments were run for 10 million generations with a burn-in of 2 million generations, and the three-molecular marker alignment was run for 30 million generations with a burn-in of 6 million generations; all alignments were analyzed using two runs of four MCMC chains sampled every 1000 generations. Maximum likelihood phylograms are presented in this paper and nodes are labelled with maximum likelihood bootstrap values (BS)/Bayesian posterior probabilities (BPP) reported as percentages. In situations where the ML and Bayesian trees are incongruent, the Bayesian node support is denoted as (-).

Hypothesis testing: Hypotheses concerning the monophyly of *Rhoicosphenia* were tested using tree likelihoods and the Approximately Unbiased (AU) test (Shimodaira 2002).

For the tests using the two and three-molecular marker alignments, an unconstrained tree (H_0) was tested against four constrained alternative topologies:

- H_{2a}: *Rhoicosphenia* is in a monophyletic clade with all members of the Heteroideae (*sensu* Grunow 1860, Hustedt 1959, Mereschkowsky 1902),
- H_{2b}: *Rhoicosphenia* is monophyletic with the clade of Heteroideae that contains *Achnanthidium*,
- H_{2c}: *Rhoicosphenia* is monophyletic with the clade of Heteroideae that does not contain *Achnanthidium*, and
- H_3: *Rhoicosphenia* and *Gomphonema* form a monophyletic group (*sensu* Van Heurck 1896, Mereschkowsky 1902).

For the tests using single molecular marker trees, the unconstrained tree (H_0) was tested against five constrained alternative topologies:

- H_{2a}: *Rhoicosphenia* is in a monophyletic clade with all members of the Heteroideae diatoms,
- H_{2b}: *Rhoicosphenia* is monophyletic with the clade of Heteroideae that contains *Achnanthidium*,
- H_{2c}: *Rhoicosphenia* is monophyletic with the clade of Heteroideae that does not contain *Achnanthidium*,
- H_{3a}: *Rhoicosphenia* and *Gomphonema* 'clade 1' (*Gomphonema* and *Gomphoneis*) form a monophyletic group, and
- H_{3b}: *Rhoicosphenia* and *Gomphonema* 'clade 2' (*G. micropus*) form a monophyletic group. Hypotheses 1 and 4 were unable to be testing using this method.

Finally, for the SSU, *rbc*L, and SSU + *rbc*L alignments, we also are testing:

- H_5: Are all 'monoraphid' diatoms monophyletic? The genera included in this test are *Achnanthes*, *Achnanthidium*, *Cocconeis*, *Lemnicola*, *Planothidium*, and *Psammothidium*. Some of the molecular marker combinations have different taxa, but are limited to these genera. And,
- H_6: Are the genera *Achnanthes* and *Mastogloia* monophyletic?

RAxML ver. 8.0.26 (Stamatakis 2014) and the graphical user interface raxmlGUI ver. 1.3.1 (Silvestro & Michalak 2012) were used to generate maximum likelihood trees from the unconstrained and constrained alignments for hypotheses 2 and 3 (A & B), using GTR+ Γ+I model. The probability that the alternative topologies were as likely as the null topology (unconstrained tree) was tested by calculating per site log likelihood values using RAxML and implementing the AU in the program CONSEL using default settings (Shimodaira & Hasegawa

2001). In CONSEL the AU test compares a hypothesized tree topology to a set of trees generated through a multi-scale bootstrap technique of per site log likelihoods. A statistically significant result, p-value less than or equal to 0.05, means that the hypothesized tree topology can be rejected, while a p-value greater than 0.05 does not allow the rejection of the hypothesized constrained tree.

Morphological analyses

The taxa, character matrix, and character states used in this analysis were published in (Cox & Williams 2006). Our analysis used 33 of the 49 taxa published in (Cox & Williams 2006) to maximize taxa shared between our morphological and molecular analyses. The characters used, as well as their coding, has been left unchanged from the original dataset (Cox & Williams 2006), but we ran all data, protoplast and frustule, together in our analysis. The explanation and coding of characters can be found in Table 5 and the taxon and character matrix is presented in Table 6.

Character #	Character	Description	State
1	Chloroplasts per cell	Two	0
		One	1
		Multiples of two	2
2	Chloroplast shape 1	Two-dimensional	0
		Three-dimensional	1
3	Chloroplast shape 2 (2-D shapes)	Incised plate (butterfly or simple H)	0
		Simple plate	1
		Double H-shape	2
4	Chloroplast shape 3 (3-D shapes)	Lobed with linking pyrenoid	1
		Variously lobed around a central axis	2
5	Chloroplast location	Along length of cell	0
		Fore and aft in cell	1
6	Position of center of plastid	Under valve	0
		Against girdle	1
		Near mid-line of cell	2
7	Pyrenoid number	More than one per chloroplast	0
		One per chloroplast	1
8	Pyrenoid position in plastid	Scattered	0
		Axial	1
		Lateral	2
9	Pyrenoid shape	Curved or rounded	0
		Rod-like (angular)	1
		Tetrahedral	2
10	Valve symmetry 1	Isopolar	0
		Heteropolar	1
11	Valve symmetry 2	Bilaterally symmetrical	0
		Dorsiventral - primary side ventral	1
		Dorsiventral - primary side dorsal	2
12	Frustule symmetry	Isovalvar	0
		Heterovalvar	1
13	Valve mantle	Uniform	0
		Stepped	1
		Notched	2

Table 5 (part 1): Characters and character states used in morphological phylogenetic analysis.

Character #	Character	Description	State
14	Striae 1	Simply areolate	0
		Chambered - external surface areolate	1
		Chambered - internal surface areolate	2
15	Striae 2	Uniseriate throughout	0
		Biseriate (at least partly)	1
		Multiseriate	2
16	Areola occlusions 1	With cribra	0
		Without cribra	1
17	Areola occlusions 2	Without hymenes	0
		With hymenes	1
18	Areola occlusions 3	With volae	0
		Without volae	1
19	Areola type	Poroid	0
		Loculate	1
20	Areola openings (external) 1	More or less circular	0
		Elongate	1
		Reniform	2
21	Areola openings (external) 2	Openings discrete	0
		Openings confluent	1
22	Areola openings (external) 3	Opening perpendicular to stria direction	1
		Opening parallel to stria direction	2
23	Girdle bands 1	With two rows of pores	0
		With one row of pores	1
		Without pores	2
24	Girdle bands 2	Pores like valve pores	0
		Pores unlike valve pores	1
25	Internal raphe sternum	Absent	0
		With central fissure	1
		With lateral fissure	2
26	Accessory rib	Absent	0
		On primary side only	1
		On primary and secondary sides	2
27	Internal central raphe fissures 1	Unilaterally deflected	0
		Straight	1
		Oppositely deflected	2
28	Internal central raphe fissures 2	Simple	0
		Hidden (+ intermissio)	1
		Helictoglossa	2

Table 5 (part 2): Characters and character states used in morphological phylogenetic analysis.

Character #	Character	Description	State
29	Internal polar helictoglossae 1	Straight	0
		Twisted	1
		Hooded	2
30	Internal polar helictoglossae 2	Discrete	0
		Fused with sternum	1
		Forming porte-crayon ending	2
31	External central raphe endings	Straight	0
		Deflected to primary side	1
		Deflected to secondary side	2
32	External raphe endings (central v. polar)	Different	0
		Similar	1
33	External polar raphe endings	Deflected to secondary side	0
		Straight	1
		Deflected to primary side	2
		Opposite	3
34	Apical pore fields	Absent	0
		At both poles	1
		At one pole	2
35	Stigmata	None	0
		One	1
		More than one	2

Table 5 (part 3): Characters and character states used in morphological phylogenetic analysis.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12
Achnanthes brevipes	0	1	?	1	1	2	1	1	0	0	0	1
Achnanthidium minutissimum	1	0	1	0	0	1	1	1	1	0	0	1
Anomoeoneis sphaerophora	1	0	2	0	0	1	1	1	0	0	0	0
Berkeleya rutilans	1	0	0	0	0	0	1	2	0	0	0	0
Caloneis amphisbaena	0	0	1	0	0	1	1	1	0	0	0	0
Caloneis silicula	1	0	0	0	0	0	0	2	0	0	0	0
Climaconeis inflexa	0	1	?	1	1	2	1	1	0	0	1	0
Climaconeis scalaris	2	1	?	1	1	2	1	1	0	0	0	0
Cocconeis placentula	1	0	1	0	0	0	1	1	1	0	0	1
Craticula ambigua	0	0	1	0	0	1	1	1	1	0	0	0
Cymbella affinis	1	0	2	0	0	1	1	1	0	0	1	0
Cymbella cymbiformis	1	0	2	0	0	1	1	1	0	0	1	0
Cymbella lanceolata	1	1	?	2	0	2	1	1	0	0	1	0
Encyonema caespitosum	1	0	2	0	0	1	1	1	0	0	2	0
Encyonema prostratum	1	0	2	0	0	1	1	1	0	0	2	0
Gomphonema acuminatum	1	0	2	0	0	1	1	1	0	1	0	0
Gomphonema parvulum	1	0	2	0	0	1	1	1	0	1	0	0
Mastogloia smithii	0	1	?	1	1	2	1	1	0	0	0	0
Navicula gregaria	0	0	1	0	0	1	1	1	1	0	0	0
Navicula tripunctata	0	0	1	0	0	1	1	1	1	0	0	0
Pinnularia gibba	0	0	1	0	0	1	1	1	0	0	0	0
Pinnularia lundii	1	0	0	0	0	0	0	2	0	0	0	0
Pinnularia viridis	0	0	1	0	0	1	?	?	?	0	0	0
Placoneis clementioides	1	1	?	2	0	2	1	1	0	0	0	0
Placoneis gastrum	1	1	?	2	0	2	1	1	0	0	0	0
Placoneis placentula	1	1	?	2	0	2	1	1	0	0	0	0
Reimeria sinuata	1	0	2	0	0	1	1	1	0	0	2	0
Rhoicosphenia curvata	1	0	2	0	0	1	1	1	0	1	0	1
Sellaphora bacillum	1	0	0	0	0	0	1	2	2	0	0	0
Sellaphora pupula	1	0	0	0	0	0	1	2	2	0	0	0
Stauroneis anceps	0	0	1	0	0	1	0	1	0	0	0	0
Stauroneis phoenicenteron	0	0	1	0	0	1	0	1	0	0	0	0
Stauroneis smithii	0	0	1	0	0	1	1	1	0	0	0	0

Table 6 (part 1): Taxon and character matrix used in morphological phylogenetic analysis.

Taxon	13	14	15	16	17	18	19	20	21	22	23	24
Achnanthes brevipes	0	0	0	0	0	1	0	0	0	0	0	0
Achnanthidium minutissimum	0	0	0	1	0	1	0	0	0	0	2	1
Anomoeoneis sphaerophora	1	0	0	1	1	1	0	0	0	0	0	0
Berkeleya rutilans	0	0	0	1	1	1	0	0	0	2	0	0
Caloneis amphisbaena	0	1	2	1	1	1	0	0	0	0	1	1
Caloneis silicula	0	1	2	1	1	1	0	0	0	0	1	1
Climaconeis inflexa	0	0	0	1	1	1	0	0	0	0	0	0
Climaconeis scalaris	0	0	0	1	1	1	0	0	0	0	0	1
Cocconeis placentula	0	0	0	1	1	1	0	1	0	2	2	1
Craticula ambigua	0	0	0	1	1	1	0	1	0	1	1	1
Cymbella affinis	1	0	0	1	0	0	0	1	0	1	1	1
Cymbella cymbiformis	1	0	0	1	0	0	0	1	0	1	1	1
Cymbella lanceolata	1	0	0	1	0	0	0	1	0	1	1	1
Encyonema caespitosum	1	0	0	1	0	1	0	1	0	1	1	1
Encyonema prostratum	1	0	0	1	0	1	0	1	0	1	1	1
Gomphonema acuminatum	1	0	1	1	0	0	0	2	0	1	1	1
Gomphonema parvulum	1	0	0	1	0	0	0	2	0	1	1	1
Mastogloia smithii	0	0	0	0	0	1	0	0	0	0	0	0
Navicula gregaria	0	0	0	1	1	1	1	1	0	1	2	1
Navicula tripunctata	0	0	0	1	1	1	1	1	0	1	2	1
Pinnularia gibba	0	1	2	1	1	1	0	0	0	0	1	1
Pinnularia lundii	0	1	2	1	1	1	0	0	0	0	1	1
Pinnularia viridis	0	1	2	1	1	1	0	0	0	0	1	1
Placoneis clementioides	1	0	0	1	0	0	0	0	0	0	1	0
Placoneis gastrum	1	0	0	1	0	0	0	0	0	0	1	0
Placoneis placentula	1	0	1	1	0	0	0	0	0	0	1	0
Reimeria sinuata	1	0	1	1	0	0	0	0	0	0	1	1
Rhoicosphenia curvata	0	0	0	1	1	1	0	1	0	1	1	0
Sellaphora bacillum	0	0	0	1	1	1	0	0	0	0	2	1
Sellaphora pupula	0	0	0	1	1	1	0	0	0	0	2	1
Stauroneis anceps	0	0	0	1	1	1	1	1	0	2	1	1
Stauroneis phoenicenteron	0	0	0	1	1	1	1	1	0	2	1	1
Stauroneis smithii	0	0	0	1	1	1	1	1	0	1	1	1

Table 6 (part 2): Taxon and character matrix used in morphological phylogenetic analysis.

Taxon	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
Achnanthes brevipes	1	0	1	0	0	0	0	0	0	0	0
Achnanthidium minutissimum	0	0	2	0	0	0	0	1	1	0	0
Anomoeoneis sphaerophora	0	0	0	0	0	0	1	0	0	0	0
Berkeleya rutilans	0	0	1	0	0	0	2	1	0	0	0
Caloneis amphisbaena	0	0	0	1	0	0	1	0	0	0	0
Caloneis silicula	0	0	0	1	0	0	1	0	0	0	0
Climaconeis inflexa	0	1	1	0	0	0	2	1	0	0	0
Climaconeis scalaris	0	2	1	0	0	0	0	1	1	0	0
Cocconeis placentula	0	0	2	0	0	1	0	0	1	0	0
Craticula ambigua	0	0	1	0	0	0	2	0	0	0	0
Cymbella affinis	0	0	0	1	2	0	1	0	0	1	1
Cymbella cymbiformis	0	0	0	1	2	0	1	0	0	1	2
Cymbella lanceolata	0	0	0	1	2	0	1	0	0	1	2
Encyonema caespitosum	0	0	0	0	0	0	1	0	0	0	0
Encyonema prostratum	0	0	0	0	0	0	1	0	0	0	0
Gomphonema acuminatum	0	0	0	0	0	0	1	0	0	2	1
Gomphonema parvulum	0	0	0	0	0	0	0	0	0	2	1
Mastogloia smithii	1	0	1	0	0	1	2	0	0	0	0
Navicula gregaria	2	1	1	0	1	1	1	0	0	0	0
Navicula tripunctata	2	1	1	0	1	1	0	0	0	0	0
Pinnularia gibba	0	0	0	1	0	0	1	0	0	0	0
Pinnularia lundii	0	0	0	0	0	0	0	0	0	0	0
Pinnularia viridis	0	0	0	1	0	0	1	0	0	0	0
Placoneis clementioides	0	0	0	0	0	0	0	0	3	0	2
Placoneis gastrum	0	0	0	0	0	0	0	0	0	0	0
Placoneis placentula	0	0	0	0	0	0	0	0	3	0	2
Reimeria sinuata	0	0	0	0	0	0	0	0	0	1	1
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	2	0
Sellaphora bacillum	0	0	0	0	0	0	1	0	0	0	0
Sellaphora pupula	0	0	0	0	0	0	1	0	0	0	0
Stauroneis anceps	0	0	1	0	0	0	2	0	0	0	0
Stauroneis phoenicenteron	0	0	1	0	0	0	2	0	0	0	0
Stauroneis smithii	0	0	1	0	?	?	0	0	0	0	0

Table 6 (part 3): Taxon and character matrix used in morphological phylogenetic analysis.

Phylogenetic analysis was performed in PAUP* 4.0b10 (Swofford 2003), and all 35 characters were unordered and equally weighted. Trees were generated using the branch-and-bound search option to determine the 200 most parsimonious trees that were then used to compute a strict consensus tree.

Results

Molecular phylogenies

In the analysis of the three-molecular marker concatenated alignment (Figure 2), both the ML and Bayesian analyses support a clade consisting of 'monoraphid' diatoms, members of the Cymbellales *sensu lato*, and *Rhoicosphenia*, to the exclusion of all other diatoms. In the ML three-molecular marker concatenated tree, *Rhoicosphenia* is not sister to *Cocconeis*, but is sister to the Cymbellales clade, with *Achnanthidium* and *Cocconeis* forming a grade basal to *Rhoicosphenia*. In the Bayesian three-molecular marker concatenated tree, *Achnanthidium* and *Rhoicosphenia* + *Cocconeis* are a 'monoraphid' grade basal to the Cymbellales.

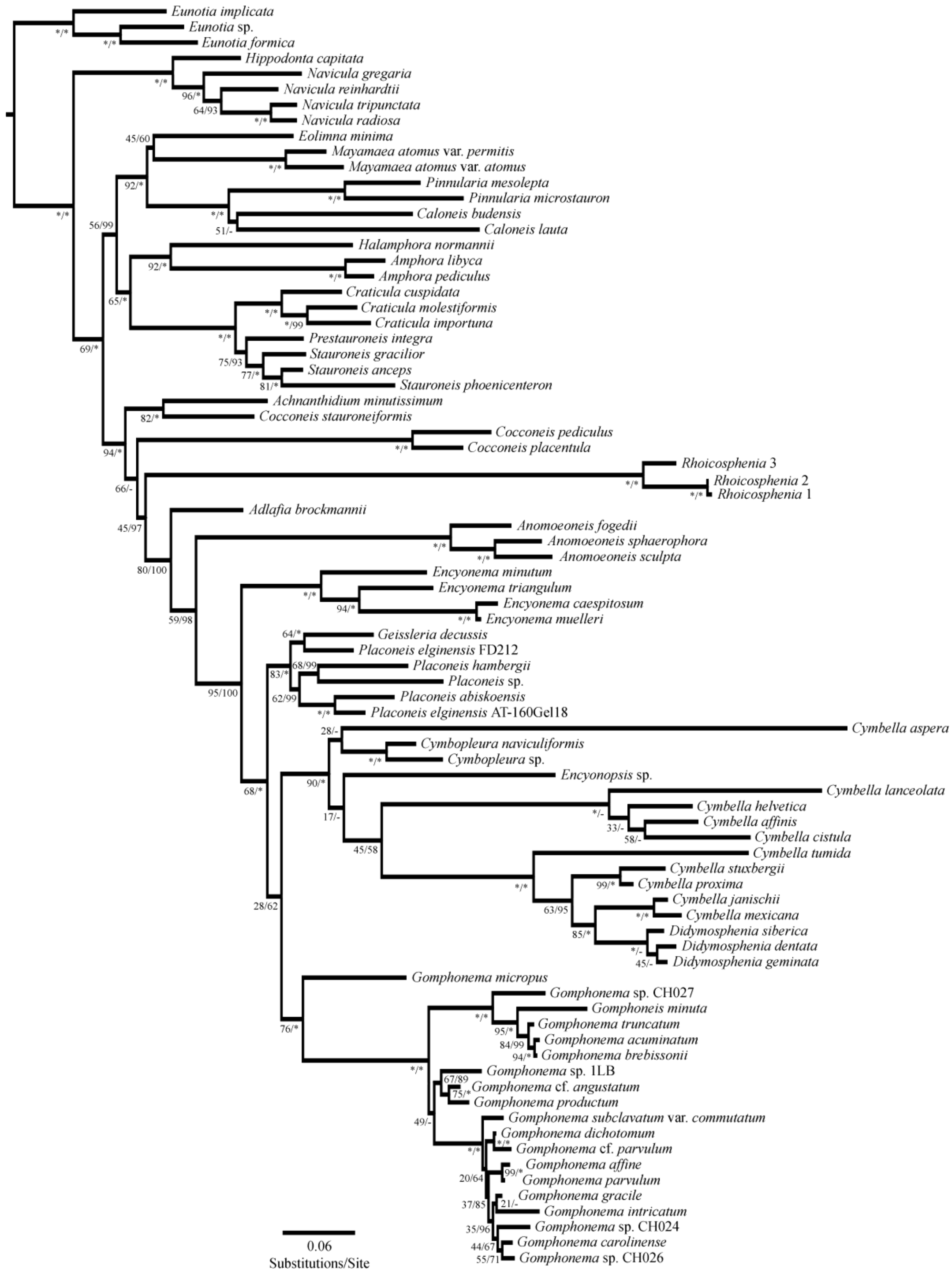


Figure 2: Maximum likelihood phylogram from three-marker concatenated alignment. Node support values are for maximum likelihood bootstrap values (500 bootstraps)/Bayesian posterior probability (as a percentage). “*” = 100, “-” = node incongruent between the two analyses.

The following files, S2–S7 Figs ("a" and "b") are tree files that can be opened with appropriate tree viewing software, such as FigTree v1.3.1, with file S#a Fig being the Maximum Likelihood tree, and S#b Fig being the Bayesian tree. These files have been uploaded to figshare (https://figshare.com) and their DOI is 10.6084/m9.figshare.3115531.

When concatenated, the two nuclear markers, SSU and LSU, show consistent topologies in both ML and Bayesian analyses (S2a,b Fig). *Rhoicosphenia* strains are monophyletic, and sister to *Anomoeoneis*, that clade is sister to a large portion of the Cymbellales, including the genera *Encyonema*, *Cymbella*, *Cymbopleura*, *Didymosphenia*, *Geissleria*, *Placoneis*, *Gomphonema*, and *Gomphoneis*. Basal to the clade containing *Rhoicosphenia* and the aforementioned genera is *Adlafia* Moser, Lange-Bertalot & Metzeltin (1998), and sister to *Adlafia* + *Rhoicosphenia* + Cymbellales is a basal grade of the 'monoraphid' genera *Achnanthidium* and *Cocconeis*.

ML and Bayesian analyses recover congruent topologies for SSU and *rbc*L when concatenated (S3a,b Fig). *Rhoicosphenia* strains are sister to *Cocconeis placentula* and *C. pediculus*, and the other 'monoraphid' taxa (*C. stauroneiformis*, *Lemnicola hungarica*, and *Achnanthidium minutissimum*) + *Rhoicosphenia* and the two *Cocconeis* are represented as a grade of taxa basal to the Cymbellales. These analyses show *Adlafia* as basal to the Cymbellales. The other 'monoraphid' taxa in these analyses, *Achnanthes sensu stricto* (four sequences), are not closely related to the previously mentioned 'monoraphid' diatoms and *Rhoicosphenia*.

LSU and *rbc*L results (S4a,b Fig) recover a monophyletic clade consisting of *Rhoicosphenia* + *Cocconeis placentula* and *C. pediculus* + *Achnanthidium minutissimum*, however, *C. stauroneiformis* is not part of that group. The clade of *Rhoicosphenia* + *C. placentula* and *C. pediculus* + *A. minutissimum* is not sister to the Cymbellales, however there is very low bootstrap support (44) for the node separating them from the intermediate clade made of biraphid naviculoid diatoms.

Both ML and Bayesian SSU analyses (S5a,b Fig) provide congruent results with the concatenated alignment that the genus *Rhoicosphenia* is basal to the Cymbellales. The SSU topology shows a well-supported (95 ML BS) lineage consisting of ‘monoraphid’ and the Cymbellales. *Cocconeis* and *Achnanthidium*, two ‘monoraphid’ genera, are non-monophyletic and are basal to a clade consisting of *Rhoicosphenia* + Cymbellales. The node where *Rhoicosphenia* splits from the Cymbellales has a bootstrap value of 45.

LSU results (S6a,b Fig) recover a topology where *Rhoicosphenia* is sister to two *Cocconeis* species, with another *Cocconeis* species sister to *Achnanthidium* and those two are not sister to *Rhoicosphenia* + *Cocconeis*. However *Rhoicosphenia* + *Cocconeis* are not sister to the Cymbellales, and are in a weakly supported (3 ML BS) clade with naviculoid diatoms. The Cymbellales clade recovered is similar to the clade in the three molecular marker and SSU analysis.

*rbc*L sequences result (S7a,b Fig) in a topology similar to the LSU analysis in that *Rhoicosphenia* is sister to *Cocconeis*. Unlike SSU, the *rbc*L phylogeny has more ‘monoraphid’ taxa (excluding *Achnanthes sensu stricto*) that form a weakly supported clade (10 ML BS) sister to the Cymbellales. Unlike LSU, *rbc*L does not result in a polytomy, but assigns branching order with *Rhoicosphenia* sister to *Cocconeis*, which together are sister to the Cymbellales.

Hypothesis testing on molecular phylogenies

Full results of hypothesis testing for all seven alignments; SSU, LSU, *rbc*L, SSU + LSU, SSU + *rbc*L, LSU + *rbc*L, and SSU + LSU + *rbc*L; can be found in Table 7.

	H_0	H_{2a}	H_{2b}	H_{2c}	H_3	H_{3a}	H_{3b}	H_5	H_6
SSU, LSU, *rbc*L	0.424	0.310	0.109	0.790	0.023*				
SSU, LSU	0.629	0.307	0.331	0.609	0.042*				
SSU, *rbc*L	0.819	0.189	0.582	0.033*	0.231			6e-5*	0.125
LSU, *rbc*L	0.367	0.257	0.843	0.199	0.040*				
SSU	0.604	0.628	0.210	0.491		0.265	0.228	6e-48*	8e-6*
LSU	0.551	0.487	0.432	0.585		0.333	0.300		
***rbc*L**	0.650	0.481	0.612	0.019*		0.225	0.188	4e-5*	0.108

Table 7: Summary of Hypothesis Testing Results. The first column states the molecular markers for the phylogeny being tested, while the first row represents the hypothesis being tested. The values in the table are the p-values from the Approximately Unbiased (AU) test (Shimodaira 2002).

In testing alternate constrained topologies against the unconstrained phylogeny, examining the three molecular marker concatenated tree, we cannot reject H_{2a}: that *Rhoicosphenia* is a Heteroideae diatom, H_{2b}: that *Rhoicosphenia* is sister to *Achnanthidium*, and H_{2c}: that *Rhoicosphenia* is sister to *Cocconeis.* The hypothesis that *Rhoicosphenia* is sister to *Gomphonema* (H_3), could be rejected ($p = 0.029$).

In the SSU + LSU analysis, we can only reject hypothesis 3, that *Rhoicosphenia* is sister to *Gomphonema* ($p = 0.042$).

For SSU + *rbc*L, we can reject H_{2c}, that *Rhoicosphenia* is sister to *Cocconeis* ($p = 0.033$), and H_5, that all 'monoraphid' diatoms are monophyletic ($p < 0.001$).

For LSU + *rbc*L, we can only reject hypothesis 3, that *Rhoicosphenia* is sister to *Gomphonema* ($p = 0.040$).

For SSU, we can reject H_5, that all 'monoraphid' diatoms are monophyletic ($p < 0.001$), and also reject H_6, that *Achnanthes sensu stricto* and *Mastogloia* are sister taxa ($p < 0.001$).

For LSU, we cannot reject any of the alternative hypotheses, $H_{2a,b,c}$ or $H_{3a,b}$.

For *rbc*L, we can reject H_{2c}, that *Rhoicosphenia* is sister to *Cocconeis* ($p = 0.019$), and H_5, that all ‘monoraphid’ diatoms are monophyletic ($p < 0.001$).

Morphological phylogeny

The strict consensus tree of the 200 trees returned from the branch-and-bound parsimony analysis was similar to the consensus tree using all data from (Cox & Williams 2006). Our tree (Figure 3) returned *Rhoicosphenia* in an unresolved polytomy of 20 taxa, however within that polytomy members of the same genus did group together. Although our tree was unable to resolve relationships with any more detail than (Cox & Williams 2006), we are still including the tree in this paper. The consistency (*CI*) and retention indices (*RI*) from our analysis, $CI = 0.4727$ & $RI = 0.7434$, are similar to those of (Cox & Williams 2006), $CI = 0.39$ & $RI = 0.77$.

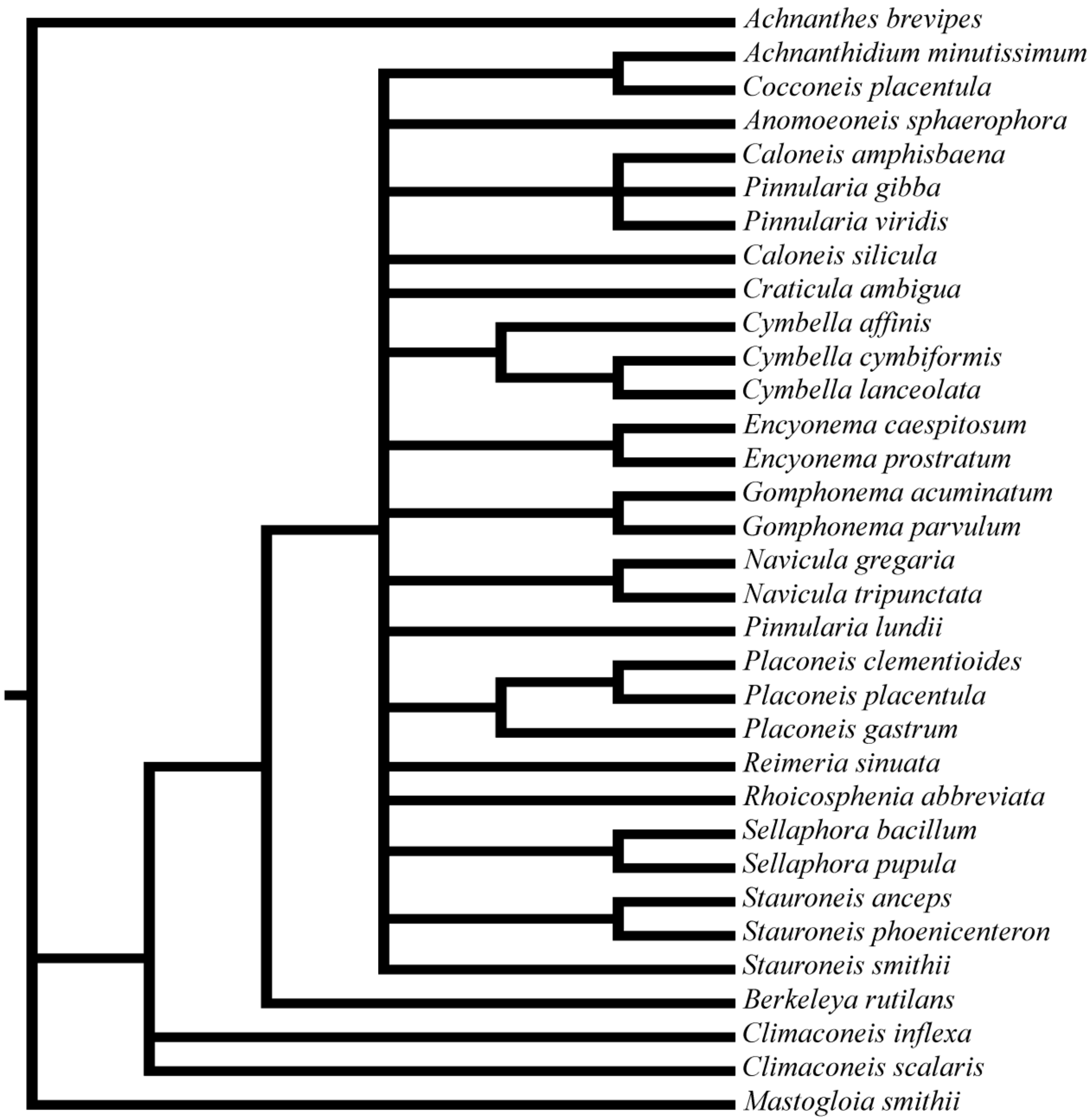


Figure 3: Strict consensus tree based on morphological characters.

Discussion

The results of the molecular analyses from this study provide insights into the evolution of the ‘monoraphid’ condition, and also lend support to the Cymbellales *sensu* Mann in (Round et al. 1990), with both of these results having implications for the systematic position of *Rhoicosphenia*. First, SSU + *rbc*L (S3a,b Fig), SSU (S5a,b Fig), and *rbc*L (S7a,b Fig), do not support a monophyletic lineage of ‘monoraphid’ diatoms of the genera *Achnanthes*, *Achnanthidium*, *Cocconeis*, *Lemnicola*, *Planothidium*, and *Psammothidium* (Table 4). Past molecular results have indicated that *Achnanthes* is more closely related to the Bacillariales than the other genera previously listed (Ruck & Theriot 2011, Kociolek et al. 2014, Stepanek & Kociolek 2014, Bruder & Medlin 2008a, Kooistra et al. 2003, Medlin & Kaczmarska 2004, Sorhannus 2004, Bruder & Medlin 2008b), however Cox (2006) hypothesized that *Achnanthes sensu stricto* and *Mastogloia* are sister taxa. Hypothesis testing for monophyly of these genera in the analyses of SSU + *rbc*L, SSU, and *rbc*L yields mixed results with SSU rejecting that relationship, while *rbc*L and SSU + *rbc*L failed to reject that relationship (Table 4). In light of these results, instead of testing the position of *Rhoicosphenia* against the non-monophyletic ‘monoraphid’ diatoms, we tested its position against the Heteroideae (Mereschkowsky 1902) consisting of the families Achnanthidiaceae (*Achnanthidium, Lemnicola, Planothidium*, and *Psammothidium*) and Cocconeidaceae (*Cocconeis*).

Our three-molecular marker analysis yields a well-supported relationship with *Rhoicosphenia* as sister to a monophyletic clade of the Cymbellales, and a grade of ‘monoraphid’ taxa including *Achnanthidium* and *Cocconeis* is sister to *Rhoicosphenia* + the Cymbellales (Figure 2). Hypothesis testing on the three-molecular marker topology rejects the hypothesis that *Rhoicosphenia* is sister to *Gomphonema*, but does not reject the hypothesis that *Rhoicosphenia* is a member of the Heteroideae. The three-gene, SSU, and *rbc*L phylogenies also support the sister relationship of the Heteroideae and the Cymbellales + *Adlafia*. This is not a novel topology, as it has been evident in other molecular analyses (Theriot et al. 2010, Ruck & Theriot 2011, Kociolek et al. 2014, Stepanek & Kociolek 2014, Bruder & Medlin 2008a), but has only been discussed in (Bruder & Medlin 2008a). The only topology rejected by hypothesis testing on the three-molecular marker analysis was the sister relationship between *Rhoicosphenia* and *Gomphonema*. The Heteroideae were monophyletic in the three-molecular marker tree, so hypotheses $H_{2b,c}$ were not tested and H_{2a} was not rejected (Table 4).

Analyses of concatenated alignments of two molecular markers generated three different topologies. The phylogeny based on SSU + LSU shows *Rhoicosphenia* as sister to *Anomoeoneis*, within the Cymbellales. This combination of molecular markers is the only one out of the seven molecular analyses to return this topology. It is interesting for two reasons. First, it is the only tree in which *Rhoicosphenia* is within, as opposed to outside the Cymbellales *sensu* Mann in (Round et al. 1990). Second, neither SSU nor LSU, when analyzed alone, return this result (S5a,b Fig, S6a,b Fig). Although parts of the tree have low support, the node that places *Rhoicosphenia* within the Cymbellales has moderate support (83 BS, 97 BPP). Hypothesis testing only rejects the sister relationship between *Rhoicosphenia* and *Gomphonema*, and fails to reject the three different hypothesis in regards to the position of *Rhoicosphenia* relative to the Heteroideae.

SSU + *rbc*L, show a sister relationship between *Rhoicosphenia* and the two freshwater *Cocconeis* species. The clade including these taxa, along with the ‘monoraphid’ genera *Lemnicola* and *Achnanthidium* is sister to a clade of *Adlafia* + Cymbellales with moderate support (71 BS, 100 BPP). *Cocconeis stauroneiformis* is not sister to the ‘monoraphid’ genera, but is basal to the other Heteroideae + Cymbellales. Hypothesis H_{2c} was rejected, meaning that even though the most likely tree places *Rhoicosphenia* and the two freshwater *Cocconeis* species as sister taxa, this relationship has very low support. This alignment allowed the testing of all 'monoraphid' genera, including *Achnanthes sensu stricto*, and the monophyly of these genera was rejected, while the hypothesis of *Achnanthes sensu stricto* as sister to *Mastogloia* was not rejected.

LSU + *rbc*L recover a moderately-supported sister relationship between *Rhoicosphenia* and *Cocconeis* (76 BS, 98 BPP), and a less well-supported sister relationship between *Rhoicosphenia* + *Cocconeis* and *Achnanthidium* (45 BS, 98 BPP), the other 'monoraphid' taxon in the analysis. However, the sister relationship between the 'monoraphid' genera and Cymbellales is not supported in this analysis and *Cocconeis stauroneiformis* does not fall with the 'monoraphid' genera. Hypothesis testing rejected the hypothesis that *Rhoicosphenia* and *Gomphonema* are sister taxa.

The single molecular marker trees generated in this study supported different hypotheses of relationships for *Rhoicosphenia*. Other studies of diatoms analyzing multiple single molecular marker and concatenated alignments (Ruck & Theriot 2011, Bruder & Medlin 2008a, Bruder & Medlin 2008b) demonstrate similar results, that is, not all single molecular marker trees recover the same tree topologies as each other or the concatenated alignment. Our single molecular marker analyses of SSU (8 BS) and *rbc*L (39 BS) suggest a weakly supported relationship between 'monoraphid' diatoms and *Rhoicosphenia*, together being sister to a moderately to poorly supported (SSU 63 BS, *rbc*L 26 BS) Cymbellales clade (S5a Fig, S7a Fig). In the SSU analysis, *Rhoicosphenia* is sister to the Cymbellales clade with a branch support of 64 (ML bootstrap). Hypothesis testing could not reject *Rhoicosphenia* as either part of the Heteroideae, or as sister to *Gomphonema*. However, the hypothesis that all 'monoraphid' diatoms are monophyletic was rejected, while the hypothesis (H_6) that *Achnanthes sensu stricto* is sister to *Mastogloia* was not rejected.

*rbc*L has weak support, 26 (ML BS), for a sister relationship between the Heteroideae and the Cymbellales, with *Rhoicosphenia* being sister to *Cocconeis* 39 (ML BS) deep within the Heteroideae. Hypothesis H_{2c} was rejected, meaning that even though the most likely trees places *Rhoicosphenia* and the two freshwater *Cocconeis* species as sister taxa, this relationship has very low support. Both the SSU and *rbc*L results support Mereschkowsky's Pyrenophoreae (Mereschkowsky 1902), based on chloroplast number and structure but including diverse valve morphologies. Hypothesis testing of all 'monoraphid' diatoms, H_5, was rejected with *rbc*L, however the hypothesis (H_6) that *Achnanthes sensu stricto* is sister to *Mastogloia* was not rejected. Unlike SSU and *rbc*L, LSU places *Rhoicosphenia* sister to *Cocconeis* with weak support 34 (ML BS), with taxa not sister to the Cymbellales. However, deeper nodes in the LSU phylogram are very weakly supported <10 (ML BS), which could be reflective of LSU being a faster evolving marker in diatoms (Alverson 2008). Our results with LSU and LSU + *rbc*L are similar to the LSU trees generated in (Bruder & Medlin 2008a, Bruder & Medlin 2008b), in that their LSU returned the most unique topology of the three single molecular marker analyses. After analyzing all trees based on single, two-, and three-molecular markers we, similar to previous investigators (Ruck & Theriot 2011, Kociolek et al. 2014, Bruder & Medlin 2008a, Bruder & Medlin 2008b), have decided to base our conclusions on the three molecular marker concatenated alignment.

With regards to morphological analysis the strict consensus tree generated from 200 most parsimonious trees produced a large polytomy of taxa, with only congeneric species within the analysis being resolved together (Figure 3). This result only differs from (Cox & Williams 2006, Figs 5–6) in that their analysis groups some genera together, within a larger unresolved polytomy. This result, when compared to (Cox & Williams 2006), indicates that our documentation and understanding of morphological characters that can inform a broad phylogeny of the raphid diatoms is currently insufficient.

In addition to the systematic position of *Rhoicosphenia*, our SSU analysis shows that the 'monoraphid' condition evolved multiple times, once in *Achnanthes sensu stricto*, and at least once in the other 'monoraphid' genera near the Cymbellales (S5a,b Fig), supporting hypotheses of Cleve (1895) and Mereschkowsky (1902). Phylogenies showing this result have been returned in all analyses that include *Achnanthes sensu stricto* and other 'monoraphid' taxa (Kociolek et al. 2014, Stepanek & Kociolek 2014 S1, Bruder & Medlin 2008a, Kooistra et al. 2003, Medlin & Kaczmarska 2004, Sorhannus 2004, Bruder & Medlin 2008b). When considering morphology, the systematic position of *Achnanthes sensu stricto* is also quite interesting. Cox (2006) suggested *Achnanthes* is closely related to *Mastogloia*, based on similarities in chloroplast, pore (cribrate), and raphe structure and cite their position in a cladistic analysis of morphology (Cox & Williams 2006). Our single molecular marker SSU, LSU and *rbc*L and multi-molecular marker analyses do not support a relationship between *Achnanthes* and *Mastogloia*, but instead place *Achnanthes* within the Bacillariales, similar to other molecular studies (Bruder & Medlin 2008a, Sorhannus 2004, Bruder & Medlin 2008b). Mereschkowsky (1902) showed the chloroplast of *Achnanthes sensu stricto* to be similar to *Hantzschia* Grunow (Grunow 1877), a genus within the Bacillariales. Placement of *Achnanthes* within the Bacillariales is problematic based on morphology, and more extensive taxon sampling in this region of the raphid diatom tree of life may help to resolve the phylogenetic position of this 'monoraphid' genus. Our molecular results, however, support the relationship between *Achnanthes* and the Bacillariales, but results of hypothesis testing do not rule out the possibility that *Achnanthes* is related to genera in the Mastogloiales. This appears to be another case, in addition to the relationships of ‘monoraphid’ diatoms and *Rhoicosphenia* with the Cymbellales, where molecular data support Mereschkowsky’s (1902) suggestion of a close relationship between taxa with diverse valve morphologies, based on chloroplast similarities.

Since the description of *Rhoicosphenia* (Grunow 1860), multiple hypotheses of its phylogenetic position have been made based on valve (Grunow 1860) and chloroplast (Mereschkowsky 1902) morphology. Detailed investigations into the valve morphology (Mann 1982a), sexual reproduction (Mann 1982b), relation to other diatom genera (Medlin & Fryxell 1984a), and initial cells and size reduction (Mann 1984, Medlin & Fryxell 1984b) were unable to support or reject any of the hypotheses from the past century as summarized in (Mann 1982a), but did support Mann’s hypothesis (H_4) that *Rhoicosphenia* belongs in an ‘enigmatic’ position (Mann 1984). Mann presented multiple lines of morphological evidence, without any formal analysis, that support the similarities of *Rhoicosphenia* to ‘monoraphid’ diatoms and *Gomphonema*, but explains their similarities as convergent evolution (Mann 1982a, Mann 1982b, Mann 1984). However, he did not question that the specific morphological traits he considers – pore occlusions, shape, heteropolarity, mucilage pads, pseudosepta, copulae, raphe structure and number, etc. – may look similar in different groups due to convergence (they are not homologous) and therefore would not be helpful in building phylogenies (Mann 1982a, Mann 1982b, Mann 1984).

Based on the concatenated three molecular marker analysis, we suggest that *Rhoicosphenia* occupies a position basal to the Cymbellales. In terms of diatom classification, with the addition of the genera *Geissleria* (Kulikovskiy et al. 2014, Nakov et al. 2014) and *Adlafia*, the Order Cymbellales *sensu* Round are a natural group – interestingly it is noted that *Adlafia* has a single chloroplast (as *Navicula brockmanii* Hustedt (Hustedt 1934) in Bruder & Medlin 2008a, Bruder & Medlin 2008b), similar to the chloroplast structure Mereschkowsky (1902) used to unite the Monoplacatae, the group in which he placed members of the Cymbellales and *Rhoicosphenia*. While our data support Mereschkowsky's Monoplacatae consisting of Heteroideae and Cymbellales, hypothesis testing rejects one specific proposal of Mereschkowsky, that is, the placement of *Rhoicosphenia* as sister to *Gomphonema* (Table 4). Our analysis supports the classification of (Round et al. 1990) that places *Rhoicosphenia* in the Cymbellales, but we add phylogenetic structure to this grouping, with *Rhoicosphenia* in a basal position to the rest of the genera in the order. The order Cymbellales would now include the genera *Adlafia*, *Anomoeoneis*, *Cymbella*, *Cymbopleura*, *Didymosphenia*, *Encyonema*, *Encyonopsis*, *Geissleria*, *Gomphoneis*, *Gomphonema*, *Placoneis*, and *Reimeria*. The relationship between diatoms in the Heteroideae and the Cymbellales (including *Rhoicosphenia*) could be assigned a Linnaean taxonomic rank of superorder named Cymbellidae that would include Achnanthidiaceae + Cocconeidaceae + *Rhoicosphenia* + Cymbellales, within the subclass Bacillariophycidae. This superorder would be very similar to Mereschkowsky's Monoplacatae, with the addition of genera that were not yet recognized in the early 20th century, and would also represent a monophyletic clade in the context of PhyloCode (de Queiroz 2012). The Cymbellales would remain an order in our classification, but two unnamed clades between the Order and Superorder ranks would also be recognized, one consisting of Cocconeidaceae + *Rhoicosphenia* + Cymbellales, the other would consist of *Rhoicosphenia* + Cymbellales. Additionally, our results support Mereschkowsky (1902) and Cox (2006) that *Achnanthes sensu stricto* should not be considered part of a monophyletic clade of 'monoraphid' diatoms, however cannot fully support or reject their specific placements of the genus. Finally, our analyses support Cleve's (1895) hypothesis that 'monoraphid' diatoms are polyphyletic. A classification scheme based on our results is presented below.

- SUPERORDER: Cymbellidae (Achnanthidiaceae + Cocconeidaceae + *Rhoicosphenia* + Cymbellales)
    - Unnamed Clade (Cocconeidaceae + *Rhoicosphenia* + Cymbellales)
        - Unnamed Clade (*Rhoicosphenia* + Cymbellales)
            - ORDER: Cymbellales (*Adlafia*, *Anomoeoneis*, *Cymbella*, *Cymbopleura*, *Didymosphenia*, *Encyonema*, *Encyonopsis*, *Geissleria*, *Gomphoneis*, *Gomphonema*, *Placoneis*, *Reimeria*, *Rhoicosphenia*)
                - Suborder: **Cymbellineae**, Suborder nov.
                    - Family: Cymbellaceae Grunow (*Adlafia*, *Anomoeoneis*, *Cymbella*, *Cymbopleura*, *Didymosphenia*, *Encyonema*, *Encyonopsis*, *Geissleria*, *Gomphoneis*, *Gomphonema*, *Placoneis*, *Reimeria*)

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CHAPTER IV

MONOPHYLY OF THE RHOICOSPHENIACEAE

Introduction

This chapter addresses the phylogeny of the diatom family Rhoicospheniaceae Chen & Zhu (1983), which is where the genus *Rhoicosphenia* is placed. Despite a rich history of both morphological and molecular diatom phylogenetics, the monophyly of the Rhoicospheniaceae has not been addressed. Despite this lack of phylogenetic analyses, in addition to *Rhoicosphenia*, ten genera have been added to the family, including *Campylopyxis* Medlin, *Chelonicola* Majewska, De Stefano & Van de Vijver, *Cuneolus* Giffen, *Epiphalaina* Holmes, Nagasawa & Takano, *Gomphonemopsis* Medlin, *Gomphoseptatum* Medlin, *Gomphosphenia* Lange-Bertalot, *Poulinea* Majewska, De Stefano & Van de Vijver, *Rhoiconeis* Grunow, and *Tursiocola* Holmes, Nagasawa and Takano (itis.gov, Guiry 2016). However, only the genera *Campylopyxis*, *Cuneolus*, *Gomphonemopsis*, *Gomphoseptatum*, and *Rhoicosphenia* were included in an earlier summary of the genera within the Rhoicospheniaceae (Round et al. 1990). When revisiting the paper that described *Epiphalaina* and *Tursiocola*, there is no mention of these genera being placed in the Rhoicospheniaceae, in fact, they were not placed in any higher level classification, but said to be ‘gomphonemoid’ (Holmes et al. 1993a). In reading the paper describing *Campylopyxis*, which was based on a species transferred out of *Rhoiconeis*, it becomes evident that while *Campylopyxis* was intended to be placed in the Rhoicospheniaceae, *Rhoiconeis* was not (Medlin 1985). The paper in which the genera *Chelonicola* and *Poulinea* were described clearly states that they are not related to the Rhoicospheniaceae, but are related to each other (Majewska et al. 2015), so it is unclear as to why they are placed in the Rhoicospheniaceae according to AlgaeBase.org (Guiry 2016).

For this analysis of the family Rhoicospheniaceae, morphological observations on the genera *Cuneolus*, *Gomphonemopsis*, *Gomphoseptatum*, and *Rhoicosphenia* – which were included in Round et al. (1990), as well as *Gomphosphenia* - in which the paper describing it clearly places it in the Rhoicospheniaceae, was completed, the other five genera were not included in the analysis because they seem to have been placed in the family in error. *Campylopyxis* was not included due to insufficient morphological data. This work was part of a collaboration that initially placed the genera *Chelonicola* and *Poulinea* in the Rhoicospheniaceae. After initial reviews, the authors were asked to include a phylogenetic study to provide evidence of that placement. I was asked to run a cladistic analysis on morphological characters and the results of the analysis suggest that the Rhoicospheniaceae is non-monophyletic. This chapter is an abridged version of that paper, all taxon images have been removed and the in text figure references refer to the original publication, the only figure that is presented in this dissertation is that of the phylogenetic tree (Figure 32). The full citation for the original publication is:

Majewska, R., Kociolek, J.P., Thomas, E.W., De Stefano, M., Santoro, M., Bolaños, F. & Van de Vijver, B. 2015. *Chelonicola* and *Poulinea*, two new gomphonemoid diatom genera (Bacillariophyta) living on marine turtles from Costa Rica. *Phytotaxa* 233 (3): 236–250. http://dx.doi.org/10.11646/phytotaxa.233.3.2

Abstract

Marine mammals such as whales and dolphins have been known for a long time to host a very specific epizoic community on their skin. Less known however is the presence of a similar community on the carapaces of sea turtles. The present study is the first describing new taxa inhabiting sea turtle carapaces. Samples, collected from nesting olive ridley sea turtles (*Lepidochelys olivacea*) on Ostional Beach (Costa Rica), were studied using light and scanning electron microscopy. Two unknown small-celled gomphonemoid taxa were analyzed in more detail and are described as two new genera, closely related to other gomphonemoid genera with septate girdle bands, such as *Tripterion*, *Cuneolus* and *Gomphoseptatum*. *Chelonicola* Majewska, De Stefano & Van de Vijver gen. nov. has a flat valve face, uniseriate striae composed of more than three areolae, simple raphe external endings, internally a siliceous flap over the proximal raphe endings and lives on mucilaginous stalks. *Poulinea* Majewska, De Stefano & Van de Vijver gen nov. has at least one concave valve, uniseriate striae composed of only two elongated areolae, external distal raphe endings covered by thickened siliceous flaps and lives attached to the substrate by a mucilaginous pad. *Chelonicola costaricensis* Majewska, De Stefano & Van de Vijver sp. nov. and *Poulinea lepidochelicola* Majewska, De Stefano & Van de Vijver sp. nov. can be separated based on stria structure, girdle structure composed of more than 10 copulae, raphe structure and general valve outline. A cladistics analysis of putative members of the Rhoicospheniaceae indicates that the family is polyphyletic. *Chelonicola* and *Poulinea* are sister taxa, and form a monophyletic group with *Cuneolus* and *Tripterion*, but are not closely related to *Rhoicosphenia*, or other genera previously assigned to this family. Features used to help diagnose the family such as symmetry and presence of septa and pseudosepta are homoplastic across the raphid diatom tree of life.

Keywords: Bacillariophyta, cladistics, Costa Rica, epizoic diatoms, marine turtles, new genus, phylogenetic analysis

Introduction

During a survey of the epizoic flora on marine olive ridley sea turtles (*Lepidochelys olivacea* Eschscholtz 1829), several small, unknown gomphonemoid diatom taxa were observed that could not be identified using the currently available (though sparse) literature about these genera. At present, several small-celled gomphonemoid genera are known from the marine environment. *Cuneolus* Giffen (1970) was described in 1970 from the African coast. Two others were split off in 1986 by Medlin & Round from the freshwater genus *Gomphonema* Ehrenberg (1832): *Gomphonemopsis* Medlin (1986) and *Gomphoseptatum* Medlin & Round (1986). An interesting feature of *Cuneolus* and *Gomphoseptatum* is the presence of septa on the valvocopulae (lacking in *Gomphonemopsis*), usually only found in araphid genera (Van de Vijver et al. 2012). Holmes et al. (1993a) described a third gomphonemoid genus bearing similar septa, living epizoically on the skin of porpoises: *Tripterion* R.W. Holmes et al. (1993a). So far, these septa-bearing genera are rather species-poor with only two species known in *Gomphoseptatum* (Medlin & Round 1986, Witkowski et al. 2000), one in *Cuneolus* (Giffen 1970, Medlin & Round 1986) and three in *Tripterion* (Holmes et al. 1993a, Holmes et al. 1993b, Fernandes & Sar 2009).

In this paper, we focus on two taxa that were recently observed living epizoically on the carapaces of sea turtles in Costa Rica. For a long time, epizoic diatom taxa were only known living either on bird feathers (Holmes & Croll 1984: *Pteroncola* R.W. Holmes & Croll 1984) and the skin and teeth of whales and dolphins (Denys 1997, Denys & Van Bonn 2001, and references therein). Apart from some occasional observations of diatom taxa in samples scraped off from whales that normally prefer other habitats, a limited number of genera seemed to be restricted to this particular habitat such as for instance *Epiphalaina* R.W. Holmes et al. (1993a), *Bennetella* R.W. Holmes (1985) and *Plumosigma* T. Nemoto (1956). Almost all recorded taxa were only known from the marine environment as most whales and dolphins are restricted to a marine life. Recently however, several new epizoic diatoms were described from a freshwater turtle in the Rio Negro (Wetzel et al. 2010, 2012), including one taxon belonging to the presumably exclusively marine ceticolous genus *Tursiocola* R.W. Holmes et al. (1993a). The discovery of these epizoic diatoms on aquatic turtles raised interesting research opportunities for the study of epizoic diatoms on other aquatic and marine animals such as marine turtles. In 2010, some preliminary results were presented during the 21st IDS conference in St. Paul (USA) (Brady 2010), although no follow-up paper on this research was published afterwards. Recently, three new *Tursiocola* taxa were described from West Indian manatees (Frankovich et al. 2015).

Based on light microscopical observations, it was almost impossible to separate the two taxa living on turtles but detailed analysis of their ultrastructure revealed important morphological differences, excluding not only conspecificity but also the position of both taxa within the same genus. Careful comparison of the features of both taxa with all small-celled gomphonemoid genera known so far (see above), led to the conclusion that both taxa cannot be attributed to either of them and should be placed in two new genera. The present paper describes therefore these two new genera *Poulinea* Majewska, De Stefano & Van de Vijver gen. nov., typified by *P. lepidochelicola* Majewska, De Stefano & Van de Vijver sp. nov., and *Chelonicola* Majewska, De Stefano & Van de Vijver gen. nov., typified by *C. costaricensis* Majewska, De Stefano & Van de Vijver sp. nov. Both genera possess a unique combination of morphological features, compared to other, similar, small-celled gomphonemoid genera.

Materials and methods

Epizoic samples used in this study were collected in October 2013 from the turtles in Ostional Beach on the Pacific coast of Costa Rica during their nesting event (*arribada*). Approximately 20 cm^2 of arbitrarily chosen carapace pieces of several olive ridley sea turtles were scraped off when the turtles came ashore to lay eggs. Although olive ridley sea turtles are a protected species, they breed with success in Ostional and are currently not endangered there. A collection of epizoic diatoms, epibionts, and ectoparasites was made by scraping individual turtle carapaces with a razor. The method is not invasive, as it is limited to the most external part of the turtle carapace scutes, and it does not harm or cause the animal suffering. All sampling procedures took place as approved by MINAE under close supervision of SINAC park rangers. All procedures involved respect the ethical standards in the Helsinki Declaration of 1975 (revised in 2000 and 2008), as well as all applicable national laws.

Samples were kept in seawater and preserved immediately with 4 % formaldehyde. In order to remove all organic material, carapace sub-samples were digested following a slightly modified method by von Stosch (Hasle & Syvertsen 1997) using a mixture of boiling concentrated acid (64 % nitric acid and 97 % sulphuric acid added at a 1:3 volume ratio). Following digestion and centrifugation, cleaned material was rinsed and diluted with deionized water. For light microscopy (LM) analysis, cleaned material was mounted permanently on glass slides using Naphrax® and observed using an Olympus BX53 microscope, equipped with Differential Interference Contrast (Nomarski) and the Olympus UC30 Imaging System. Samples and slides are stored at the Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, II University of Naples, and the BR-collection, property of the Belgian federal government and given in permanent loan to the Botanic Garden Meise

(Belgium). For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered through a 1-µm Isopore™ polycarbonate membrane filter (Merck Millipore).

The second part of the collected material was cut into ca. 2 cm^2 squares and dehydrated by immersion in alcohol series at increasing gradation (20, 30, 40, 50, 60, 70, 80, 90, 95, 100 % alcohol solutions in distilled water). Subsequently, carapace pieces were treated with a Critical Point Drier (K850 EMITECH), placed on aluminum stubs with carbon tape. The stubs were sputter-coated with a Gold-Palladium layer of 20 nm and studied in a ZEISS Supra 40 SEM microscope at 5 kV (Centro Grandi Apparecchiature, II University of Naples, Naples, Italy). Diatom terminology follows Ross et al. (1979), Medlin & Round (1986), Round et al. (1990), Fernandez & Sar (2009) and Van de Vijver et al. (2012). The morphology of the new taxa has been compared with the ultrastructure of known epizoic species described worldwide (Nemoto 1956, Giffen 1970, Medlin & Round 1986, Holmes et al. 1993a, Holmes et al. 1993b, Witkowski et al. 2000, Fernandez & Sar 2009).

The discriminating features of both new taxa are hardly discernible in the light microscope making it impossible to separate both taxa in LM. Scanning electron microscopy was essential to clarify the morphological characteristics of both taxa. Therefore the scanning electron microscopy stub was designated as holotype for both new taxa.

The phylogenetic position of the taxa considered herein was determined through a cladistic analysis of morphological features. The 23 taxa included other genera assigned to the Rhoicospheniaceae by Round et al. (1990), Lange-Bertalot (1995), and Fernandes & Sar (2009), as well as taxa thought to be close allies of this group. These include representatives of the 'monoraphid' Achnanthidiaceae and Cocconeidaceae) and the Cymbellales, shown to be close allies of *Rhoicosphenia* (Jones et al. 2005, Nakov et al. 2014, Thomas et al. 2016). Based on previous phylogenetic analyses of the raphid diatoms, *Achnanthes brevipes* Agardh (1824) and *Mastogloia smithii* Thwaites in lit. ex W.Smith (1856), are both positioned as early branches in the naviculoid diatoms (Ruck & Theriot 2011; Kociolek et al. 2013) were identified as the outgroups for this analysis. The analysis included 28 characters and character state definitions for valve morphology as suggested by Cox & Williams (2006) and Kociolek & Stoermer (1993) and are presented in Table 1. The data matrix of terminal taxa, characters and character states is found in Table 2.

Character #	Character	Description	State
10	Valve symmetry 1	Isopolar	0
		Heteropolar	1
11	Valve symmetry 2	Bilaterally symmetrical	0
		Dorsiventral - primary side ventral	1
		Dorsiventral - primary side dorsal	2
12	Frustule symmetry	Isovalvar	0
		Heterovalvar	1
14	Striae 1	Simply areolate	0
		Chambered - external surface areolate	1
		Chambered - internal surface areolate	2
15	Striae 2	Uniseriate throughout	0
		Biseriate (at least partly)	1
		Multiseriate	2
16	Areola occlusions 1	With cribra	0
		Without cribra	1
17	Areola occlusions 2	Without hymenes	0
		With hymenes	1
18	Areola occlusions 3	With volae	0
		Without volae	1
19	Areola type	Poroid	0
		Loculate	1
20	Areola openings (external) 1	More or less circular	0
		Elongate	1
		Reniform	2
21	Areola openings (external) 2	Openings discrete	0
		Openings confluent	1
22	Areola openings (external) 3	Opening perpendicular to stria direction	1
		Opening parallel to stria direction	2
23	Girdle bands 1	With two rows of pores	0
		With one row of pores	1
		Without pores	2
24	Girdle bands 2	Pores like valve pores	0
		Pores unlike valve pores	1
25	Internal raphe sternum	Absent	0
		With central fissure	1
		With lateral fissure	2

Table 1 (part 1): Characters and character states used in morphological phylogenetic analysis.

Character #	Character	Description	State
26	Accessory rib	Absent	0
		On primary side only	1
		On primary and secondary sides	2
27	Internal central raphe fissures 1	Unilaterally deflected	0
		Straight	1
		Oppositely deflected	2
28	Internal central raphe fissures 2	Simple	0
		Hidden (+ intermissio)	1
		Helictoglossa	2
29	Internal polar helictoglossae 1	Straight	0
		Twisted	1
		Hooded	2
30	Internal polar helictoglossae 2	Discrete	0
		Fused with sternum	1
		Forming porte-crayon ending	2
31	External central raphe endings	Straight	0
		Deflected to primary side	1
		Deflected to secondary side	2
32	External raphe endings (central v. polar)	Different	0
		Similar	1
33	External polar raphe endings	Deflected to secondary side	0
		Straight	1
		Deflected to primary side	2
		Opposite	3
34	Apical pore fields	Absent	0
		At both poles	1
		At one pole	2
35	Stigmata	None	0
		One	1
		More than one	2
36	Septa	Absent	0
		Present	1
37	Pseudosepta	Absent	0
		Present	1
38	Growth Form	Free-living	0
		Attached without a stalk	1
		Attached with a stalk	2
		Tube dwelling	3

Table 1 (part 2): Characters and character states used in morphological phylogenetic analysis.

Taxon	10	11	12	14	15	16	17	18	19	20	21	23	24
Achnanthes brevipes	0	0	1	0	0	0	0	1	0	0	0	0	0
Achnanthidium minutissimum	0	0	1	0	0	1	0	1	0	0	0	2	1
Caloneis amphisbaena	0	0	0	1	2	1	1	1	0	0	0	1	1
Chelonicola	1	0	0	0	0	1	1	1	0	0	0	1	1
Cocconeis placentula	0	0	1	0	0	1	1	1	0	1	0	2	1
Craticula ambigua	0	0	0	0	0	1	1	1	0	1	0	1	1
Cuneolus	1	0	1	0	0	1	0	1	0	0	0	1	0
Cymbella affinis	0	1	0	0	0	1	0	0	0	1	0	1	1
Encyonema caespitosum	0	2	0	0	0	1	0	1	0	1	0	1	1
Gomphonema acuminatum	1	0	0	0	1	1	0	0	0	2	0	1	1
Gomphonemopsis	1	0	0	0	0	1	1	1	0	1	0	1	1
Gomphoseptatum	1	0	0	0	0	1	0	1	0	1	0	1	1
Gomphosphenia	1	0	0	0	0	1	1	1	0	1	0	1	1
Mastogloia smithii	0	0	0	0	0	0	0	1	0	0	0	0	0
Navicula gregaria	0	0	0	0	0	1	1	1	1	1	0	2	1
Pinnularia gibba	0	0	0	1	2	1	1	1	0	0	0	1	1
Placoneis placentula	0	0	0	0	1	1	0	0	0	0	0	1	0
Poulinea	1	0	0	0	0	1	1	1	0	1	0	1	0
Reimeria sinuata	0	2	0	0	1	1	0	0	0	0	0	1	1
Rhoicosphenia curvata	1	0	1	0	0	1	1	1	0	1	0	1	0
Sellaphora pupula	0	0	0	0	0	1	1	1	0	0	0	2	1
Stauroneis anceps	0	0	0	0	0	1	1	1	1	1	0	1	1
Tripterion	1	0	0	0	0	1	0	1	0	0	0	1	0

Table 2 (part 1): Taxon and character matrix used in morphological phylogenetic analysis.

Taxon	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**
Achnanthes brevipes	1	0	1	0	0	0	0	0	0	0	0	0	0
Achnanthidium minutissimum	0	0	2	0	0	0	0	1	1	0	0	0	0
Caloneis amphisbaena	0	0	0	1	0	0	1	0	0	0	0	0	0
Chelonicola	1	0	1	1	1	1	2	0	2	0	0	1	1
Cocconeis placentula	0	0	2	0	0	1	0	0	1	0	0	0	0
Craticula ambigua	0	0	1	0	0	0	2	0	0	0	0	0	0
Cuneolus	1	0	0	0	0	1	0	0	1	0	0	1	1
Cymbella affinis	0	0	0	1	2	0	1	0	0	1	1	0	0
Encyonema caespitosum	0	0	0	0	0	0	1	0	0	0	0	0	0
Gomphonema acuminatum	0	0	0	0	0	0	1	0	0	2	1	1	1
Gomphonemopsis	0	0	1	0	0	0	0	0	1	0	0	0	0
Gomphoseptatum	1	0	0	2	0	1	1	0	1	2	0	1	1
Gomphosphenia	0	0	0	0	0	0	0	1	1	0	0	0	0
Mastogloia smithii	1	0	1	0	0	1	2	0	0	0	0	0	0
Navicula gregaria	2	1	1	0	1	1	1	0	0	0	0	0	0
Pinnularia gibba	0	0	0	1	0	0	1	0	0	0	0	0	0
Placoneis placentula	0	0	0	0	0	0	0	0	3	0	2	0	0
Poulinea	2	0	1	1	0	1	2	0	2	0	0	1	1
Reimeria sinuata	0	0	0	0	0	0	0	0	0	1	1	0	0
Rhoicosphenia curvata	0	0	0	0	0	0	0	0	0	2	0	1	1
Sellaphora pupula	0	0	0	0	0	0	1	0	0	0	0	0	0
Stauroneis anceps	0	0	1	0	0	0	2	0	0	0	0	0	0
Tripterion	1	0	1	0	0	1	1	0	0	2	0	1	0

Table 2 (part 2): Taxon and character matrix used in morphological phylogenetic analysis.

A Branch-and-Bound Search for most parsimonious trees was completed in PAUP*4.0a146 (Swofford 2003) and character state data were analyzed as unordered and unweighted. The four most equally parsimonious trees were used to build a strict consensus tree which is presented in Figure 32.

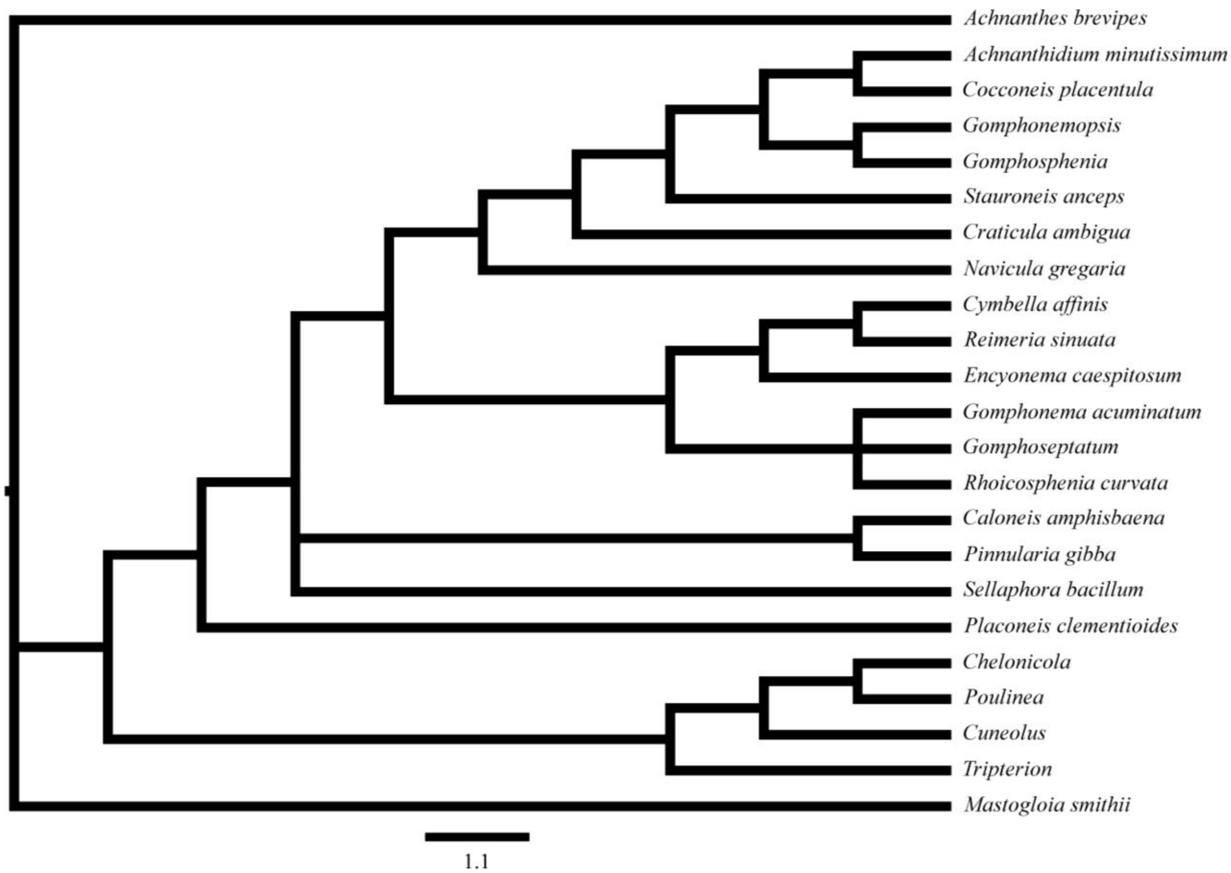

Figure 32: Strict consensus tree of taxa used to study monophyly of Rhoicospheniaceae.

Observations

Division Bacillariophyta

Class Bacillariophyceae Haeckel emend. Medlin & Kaczmarska 2004

Subclass Bacillariophycidae D.G. Mann in Round et al. 1990

Genus *Chelonicola* Majewska, De Stefano & Van de Vijver gen. nov.

Description: Frustules isovalvar, wedge-shaped in girdle view. Girdle composed of a large number (>10) of open, perforated bands of equal width. Valvocopula bearing a septum at the headpole and the second copula with a septum at the footpole. Valves heteropolar with a broadly rounded headpole and an acutely rounded footpole. Pseudosepta and apical pore field absent. Raphe straight to very weakly curving. Proximal raphe endings unilaterally weakly deflected towards the primary side. Distal raphe fissures elongated, deflected to the secondary side, continuing on both poles shortly onto the mantle. Internal proximal raphe endings covered by silica flap and distal raphe endings straight, terminating on weakly developed helictoglossae. Striae uniseriate, composed of several small, rounded areolae. Areolae internally occluded by hymenes.

Etymology: The generic name refers to the epizoic habitat where it was found: living (Latin: -*cola* = living on) on the carapaces of sea turtles (Latin: *Chelonia* = turtle)

Type species: *Chelonicola costaricensis* Majewska, De Stefano & Van de Vijver sp. nov.

***Chelonicola costaricensis* Majewska, De Stefano & Van de Vijver sp. nov.** (Figs 1–12)

Type: Costa Rica. Olive ridley sea turtle, 9º59’23.7”N/85º41’52.6´”W, leg. M. de Stefano, coll. date 27/10/2013 (holotype, stub BR–4420).

Description: Frustules wedge-shaped in girdle view showing conspicuous septa at both poles. Valves small, heteropolar, typically clavate with a broadly rounded, non-protracted headpole and an acutely terminating footpole. Septa visible in LM and SEM on both poles. Valve dimensions (n=50): length 6–17.5 µm, width 1.7–3.1 µm. Axial area very narrow, not discernible in LM. Central area very small. Raphe filiform, straight with simple, indistinct proximal raphe endings. Distal raphe endings not discernible in LM. Striae almost parallel throughout the entire valve, very faintly visible in LM, 36–47 in 10 µm.

Scanning Electron Microscopy (Figs 1–12): Frustules isovalvar, clavate in girdle view, attached by the footpole on short mucilaginous stalks (Fig. 3). Valve face flat in both valves with a clear angle to the very shallow mantle (Figs 3 & 7). The mantle is equally high in its distal and proximal part but larger in the central part (Fig. 7). Pseudosepta absent (Fig. 9, 10 & 12). Axial area very narrow, linear (Figs 4–6). Central area very small, bordered on one or both sides by one slightly shortened central stria (Fig. 4–7). Fascia never present (Fig. 5). External raphe branches almost straight to very weakly curving (Figs 4–6). External proximal raphe endings slightly expanded, unilaterally weakly deflected (Fig. 5). External distal raphe fissures elongated, weakly deflected, continuing shortly onto the mantle on both poles (Figs 6 & 7). Striae uniseriate, equally spaced throughout the entire valve, composed of a series of 3–5 slightly transapically elongated areolae (Figs 4–7). Areolae bordering the axial area being the largest (Fig. 6). Striae continuing without interruption onto the shallow mantle (Figs 4–7). Apical pore field absent on both poles (Figs 4, 6 & 8). Internally, raphe straight to weakly curved, positioned asymmetrically in a raised raphe sternum (Figs 9–12). Primary side of the sternum thickened, opening the raphe in a lateral position (Figs 9–12). Proximal raphe endings covered by a silica flap and distal raphe endings straight, terminating on weakly developed helictoglossae (Figs 9–12). Areolae internally slightly sunken between thickened interstriae, covered by hymenes (Figs 11 & 12). Cingulum composed of a large number (up to 12) of open copulae, each with one row of apically elongated, slit-like poroids in the advalvar position (Figs 3, 4 & 7). First band, the valvocopula, with a small, but distinct septum at the head pole (Fig. 9). Second copula with a small septum at the footpole (Fig. 10). Other copulae lacking a septum.

Etymology: The specific epithet refers to the geographical locality, Costa Rica, where the species was first observed.

Division Bacillariophyta

Class Bacillariophyceae Haeckel emend. Medlin & Kaczmarska 2004

Subclass Bacillariophycidae D.G.Mann in Round et al. 1990

Genus *Poulinea* Majewska, De Stefano & Van de Vijver *gen. nov.*

Description: Frustules wedge-shaped to rectangular in girdle view. One valve typically concave while other straight. Girdle composed of a large number (>10) of open, perforated bands of different width with occasionally two irregular rows of poroids. Valvocopula bearing a septum at the headpole and the second copula with a septum at the footpole. Valves heteropolar with a broadly rounded headpole and a more acutely rounded footpole. Pseudosepta absent. Apical pore field absent but several more closely-spaced areolae surrounding the distal raphe endings. Raphe straight to very weakly curving. Raphe branch in the headpole shorter than in the footpole. Proximal raphe endings straight to weakly unilaterally deflected. External distal raphe fissures elongated, deflected, located in a shallow groove, covered by a large silica flap extending from both valve apices. Internal proximal raphe endings covered by a silica flap and distal raphe endings straight, terminating on weakly developed helictoglossae. Striae uniseriate, composed of two elongated areolae, clearly separated by the valve face/mantle junction. Areolae occluded in the areolar canal by hymenes.

Etymology: The genus is named in honour of our colleague and dear friend Dr. Michel Poulin (Canadian Museum of Nature, Ottawa, Canada) in recognition of his important research on marine diatoms.

Type species: *Poulinea lepidochelicola* Majewska, De Stefano & Van de Vijver sp. nov.

***Poulinea lepidochelicola* Majewska, De Stefano & Van de Vijver sp. nov.** (Figs 13–31)

Type: Costa Rica. Olive ridley sea turtle, 9º59’23.7”N/85º41’52.6´”W, leg. M. de Stefano, coll. date 27/10/2013 (holotype, stub BR–4421).

Description: Frustules wedge-shaped in girdle view showing conspicuous septa at both poles. One valve slightly concave while other valve flat. Valves small, heteropolar, typically clavate with acutely rounded, non-protracted headpole and footpole. Septa visible in LM and SEM on both poles. Valve dimensions (n=50): length 5.2–10 µm, width 1.6–2.8 µm. Axial area very narrow, not discernible in LM. Central area forming a wide fascia. Raphe filiform, curved with expanded proximal raphe endings. Distal raphe endings not discernible in LM, typically covered by a silica flap on both poles, only visible in SEM. Striae weakly radiate near the central area, almost parallel throughout the rest of the valve, very faintly visible in LM, 25–36 in 10 µm, composed of only two, transapically elongated areolae, only discernible in SEM.

Scanning Electron Microscopy (Figs 13–31): Frustules heterovalvar, wedge-shaped in girdle view (Fig. 15), attached by the footpole to the substrate by a mucilaginous pad (Figs 13, 14). Valve face flat in one valve and slightly concave in the other (Fig. 15). Valve face gently sloping towards the mantle margin (Fig. 26). Mantle height largest near the valve middle becoming shallower towards both poles (Figs 15, 16 & 26). Pseudosepta absent (Figs 27–29). Axial area narrow, linear, narrowing towards the apices (Fig. 21). Central area small, forming a rectangular fascia that widens towards the valve margins (Figs 21, 24, 26, 27). Occasionally shortened striae present in the central area (Figs 15, 16 & 21). External raphe branches differing in length with branch in upper half (headpole) shorter than in lower halve of the valve (Figs 16 & 21). Branches almost straight to curving (Fig. 21). External proximal raphe endings spatulate, unilaterally weakly deflected (Fig. 24). Distal raphe fissures elongated, unilaterally bent, terminating near the valve poles, covered on the headpole and footpole by silica flaps, conspicuously thickened on the footpole (Figs 21–23). Striae uniseriate, equally spaced on most of the valve, but somewhat denser near the poles (Figs 21–23), composed of 1–2 (very rarely 3, Figs 27–29) transapically elongated areolae (Fig. 25). Both rows of areolae separated by a larger hyaline area, formed by the valve face/mantle junction (Figs 25 & 26). Apical pore field absent on both poles, but one series of elongated areolae surrounding the distal raphe ending present at the footpole (Fig. 23). Internally, raphe straight, located on a raised raphe sternum (Fig. 27). Proximal raphe endings covered by a silica flap (Figs 30, 31). Evident in oblique view, proximal raphe endings terminating on a slightly raised central nodule (Fig. 31). Distal raphe endings straight, terminating on weakly developed helictoglossae (Figs 28 & 29). Areolae internally slightly sunken between interstriae, covered by hymenes located in the middle of the areolar canal (Figs 28–30). Cingulum composed of a large number (up to 12) of open copulae (Fig. 20), each with one row of apically elongated, slit-like poroids in the advalvar position (Figs 15–18). Near the footpole, a double row of poroids often present on the copulae (Fig. 17, arrow). First band, the valvocopula, with a small, but distinct septum at the head pole (Figs 19 & 20). Second copula with a small septum at the footpole (Fig. 19). Other copulae lacking a septum.

Etymology: The specific epithet *lepidochelicola* refers to the habitat of the new species, living (Latin -*cola*) on *Lepidochelys olivacea*.

Phylogenetic analysis

A total of four most parsimonious trees of 97 steps was recovered in the cladistics analysis. From these four trees, a strict consensus tree was computed and is presented in Figure 32 and had a consistency index of 0.4433 and retention index of 0.5970.

The strict consensus tree shows a monophyletic clade with *Chelonicola* sister to *Poulinea*. This group is sister to *Cuneolus*, and together that group of three genera is sister to *Tripterion*. Other taxa suggested to be part of the Rhoicopheniaceae are found in widely divergent places in the tree, either sister to gomphonemoid diatoms (*Rhoicosphenia* and *Gomphoseptatum*) or 'monoraphid' diatoms (*Gomphonemopsi*s and *Gomphosphenia* are sisters and then related to a clade of *Achnanthidium* and *Cocconeis*).

Discussion

A comparison of morphological features of both new taxa (*Chelonicola costaricensis* and *Poulinea lepidochelicola*) with that of similar known small-celled gomphonemoid genera including *Gomphonemopsis, Gomphosphenia*, *Gomphoseptatum*, *Tripterion*, and *Cuneolus* (Table 1) reveals important combinations of differences, justifying the description of the two new genera. These significant morphological differences include the presence/absence of septate girdle bands, striae structure, the presence/absence or development of apical pore fields, raphe structure, and cingulum structure. *Rhoicosphenia* Grunow (1860) is similar to these genera in having pseudosepta and valves bent along the transapical axis, but it is excluded from further comparisons because the reduced raphe structure and distinct striae structure clearly differentiate this genus from the others.

Only a few genera show the presence of septate girdle bands. Van de Vijver et al. (2012) discussed the structure of septate girdle bands in both raphid and araphid diatoms and concluded that in most cases the term 'septum' was erroneously used in raphid diatom morphology, reducing the number of raphid genera with a septum to only a handful: *Cuneolus*, *Gomphoseptatum*, *Tripterion*, *Chelonicola* and *Poulinea* all possess one (*Cuneolus*) or two septate girdle bands (Round et al. 1990, Holmes et al. 1993a, present study). Some *Rhoicosphenia* taxa possess siliceous flaps on their valvocopula (septa-like structure) although real septa in the sense of araphid diatoms never have been observed (E. Thomas, personal communication) *Gomphonemopsis* and *Gomphosphenia* do not possess septa. *Cuneolus*, *Rhoicosphenia* and *Gomphoseptatum* have pseudosepta at one (*Gomphoseptatum*) or two (*Cuneolus*, *Rhoicosphenia*) poles (Round et al. 1990), contrary to both new genera that lack pseudosepta.

Based on stria structure, two separate groups of genera can be formed. A first group contains those genera having striae with three or more areolae: *Cuneolus*, *Rhoicosphenia*, *Tripterion*, and *Chelonicola*, whereas a second group is formed by all gomphonemoid genera with maximum of two, rarely three, areolae per stria: *Gomphoseptatum*, *Gomphosphenia*, and *Gomphonemopsis*, and *Poulinea* (Medlin et al. 1986, Round et al. 1990, present study). *Cuneolus* can be further separated based on differences in the structure of the internal proximal raphe endings (being clearly hooked, not covered by siliceous flap) and a much lower number of girdle bands (Medlin et al. 1986, Round et al. 1990). *Gomphoseptatum* differs in the presence of a well-developed apical pore field at the footpole (absent in both new taxa), the presence of short projections constricting the areolae into several sections (see Round et al. 1990, pg. 477, Fig. f) (never observed in both new taxa), a girdle containing a lower number of copulae, simple internal proximal raphe endings, and the lack of a siliceous flap (Medlin et al. 1986, Round et al. 1990). It should be noted however that the presence of the siliceous flap on the central nodule is not a very discriminating feature as in several larger genera such as *Pinnularia* or *Cymbella*, species can be found with and without this siliceous covering (Round et al. 1990) making this feature less important in separating both new taxa from either *Cuneolus* or *Gomphoseptatum*.

Based on the morphological comparison, only *Tripterion* shows sufficient morphological similarity with the two new taxa to warrant further morphological analysis. Three species of *Tripterion* are known: *T. kalamensis* (Holmes et al. 1993a), *T. philoderma* Holmes et al. (1993b) and *T. margaritae* (Frenguelli & Orlando 1958). *Tripterion philoderma* is most similar to *P. lepidochelicola*, with both having septate girdle bands, fascia, radial to parallel (at apices) striae, trans-apically elongated punctae, and closely-spaced punctae in rows at the footpole (Table 1). However, some important differences can be noted, seen particularly, in the two other *Tripterion* species. *Tripterion kalamensis* and *T. margaritae* possess circular to oval punctae in greater number in each stria (Holmes et al. 1993a, Fernandes & Sar 2009). All *Tripterion* species have at least three, usually 4–5 areolae per stria, a feature never observed in *Poulinea* but present in *Chelonicola*. All *Tripterion* species (Holmes et al. 1993a, Holmes et al. 1993b, Fernandes & Sar 2009) show a clear increase in stria density near the footpole compared to the valve central area. In both new taxa, this was not observed. Moreover, the areolae become smaller in *Tripterion* close to the footpole (Holmes et al. 1993a), whereas in both new taxa, the shape of the areolae does not seem to change. In *T. margaritae* the areolae near the footpole almost form an apical pore field (Fernandes & Sar 2009, Figs 36 & 37). In *T. philoderma* rows of closely-spaced elongate punctae, resembling an apical pore field, are present along the edge of the valve at the apices (Holmes et al. 1993b, Fig. 6). Similar arrangements of punctae at the apices was not found in *C. costaricensis,* but a single row of closely spaced punctae was observed at the footpole of *P. lepidochelicola*. The external raphe structure of *Tripterion* resembles *Poulinea* in having a thickened siliceous flap on the distal raphe fissures but differs from *Chelonicola* that is lacking this feature (Holmes et al. 1993b, Pl. 2, Figs 1 & 2). Both new genera also differ from *Tripterion* in the structure of the internal proximal raphe endings since the latter lacks a siliceous flap over the endings (see for instance Fernandes & Sar 2009, Fig. 37), typical for both *Chelonicola* and *Poulinea*. *Tripterion* has a much lower number of copulae in its girdle, whereas both new genera have at least 10 separate girdle elements.

In terms of the systematic placement of the two new genera described here, cladistic analysis shows that they are closely allied to one another, as sister taxa within a branch of naviculoid diatoms that includes also *Cuneolus* and *Tripterion*. Synapomorphies to support this overall clade include heteropolar valve symmetry and presence of septa (characters 1, 26, respectively) diagnose this clade. The synapomophies of external polar raphe ends being straight and presence of pseudosepta (characters 23, 27, respectively) suggest *Cuneolus* is more closely related to *Poulinea* and *Chelnicola*, the latter two sharing presence of hymenate occlusions, the deflection of the proximal and polar raphe ends (characters 21, 23) as synapomorphies. Features thought to be shared amongst members of the Rhoicospheniaceae, such as heteropolar symmetry, and presence of septa-like structures and pseudosepta are seen to be homoplasic in this lineage plus gomphonemoid diatoms and in the group closely allied with the ‘monoraphid’ diatoms.

Based on the results presented here, the Rhoicospheniaceae, as circumscribed originally by Chen and Zhu (1983) to accommodate the unique features of *Rhoicosphenia*, may be quite limited in the taxa it represents, possibly containing only *Rhoicosphenia* and *Gomphoseptatum* which are more closely related to the Cymbellales than other taxa considered here. Other putative members of the family are not shown to be closely related, however, there does appear to be a monophyletic clade of the genera of epizooic and attached diatoms, including *Poulinea* and *Chelonicola*, for which some higher Linnaean category might be proposed in the future.

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CHAPTER V

EXPLORING *RHOICOSPHENIA* SPECIES BOUNDARIES

Introduction

Rhoicosphenia Grunow is frequently observed in freshwater diatom communities sampled from streams and rivers across the United States. In many of the streams that *Rhoicosphenia* is found are part of long-term ecological monitoring projects providing data to understand the ecology of the various species (ANS et al. 2011–2016). Historically, species diversity from across the US has been limited to *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot and therefore this species has been considered to be cosmopolitan in its biogeography and broadly tolerant of ecological conditions (ex. pH, phosphorus, conductivity, etc.) (Lowe 1974). Unlike other commonly reported diatom species, such as *Achnanthidium minutissimum* (Kützing) Czarnecki, the ubiquity of *R. abbreviata* has remained largely unquestioned (Kociolek et al. 2015b). Observations of *A. minutissimum* in the Upper Great Lakes had been categorized as "tolerant of nutrient addition" and "abundant in more oligotrophic regions", however, no such statements were made of *R. curvata* (=*R. abbreviata*) which was also "common" or "abundant" in the same study (Stoermer 1980). A study on *A. minutissimum* and morphologically similar taxa, all of which are regarded as poor in morphological characters to distinguish among them, also studied ecological parameters of their habitats to aid in species delimitation (Potapova & Hamilton 2007). Results of this study indicated that while neither morphology nor ecology could on their own fully inform taxon identifications, their use in concert could delimit species. One difference between the genera *Achnanthidium* and *Rhoicosphenia* in regards to US taxa is that *Achnanthidium* is far more species rich than *Rhoicosphenia* which, prior to species discoveries in California (Thomas & Kociolek 2015), was recognized as only one species. The main distinction is that the *Achnanthidium* species were described from European localities, and this European flora was used to identify species within the US. It wasn't until 2007 when Potapova & Hamilton studied the ecological preferences of these various species. Ecological preferences of US *Achnanthidium* species were not investigated until Potapova & Hamilton (2007) conducted an in-depth look at the US flora. While the US flora of *Achnanthidium* wasn't modified in regards to European taxa, data about habitat preferences of species found in the US was added. However, in the case of *Rhoicosphenia*, ecology can be used to aid in the delimitation of species that will be described from the US in the future.

The broad morphological variation shown from populations of what has been reported as *R. abbreviata* (and *R. curvata*) (Wolle 1890, Boyer 1927, Sovereign 1958, Lowe 1970, Patrick & Reimer 1975, Czarnecki & Blinn 1977, Czarnecki & Blinn 1978, Czarnecki 1979, Benson & Rushforth 1975, Lawson & Rushforth 1975, Patrick & Reimer 1975, Clark & Rushforth 1977, Grimes & Rushforth 1982, Reavie & Smol 1998) reveal undescribed morphologies that have substantial variation to be described as new diatom species. A recent investigation into *Rhoicosphenia* diversity from streams in California revealed three new freshwater *Rhoicosphenia* (Thomas & Kociolek 2015) and several new morphologies from streams across the US that are in the process of being described (Thomas *in prep*). While approximately 180 diatom species are described per year (Julius 2007) only seven *Rhoicosphenia* have been described since 1970 or approximately 0.08% of the new species described over the past 46 years. Diatom taxonomists relied upon, and continue to use, a morphological species concept when describing new species –

that is, if two populations have features that look different, they are considered to be two independent species (Round et al. 1990). Descriptions of diatom species commonly discuss in detail the size (length, breadth) and other morphological characters, such as number and position of raphe branches, number of striae per unit length (usually 10 µm), shape and distribution of openings on valve face, and other genus or taxon specific traits. These differences can be ‘large’, such as differences in valve shape or disparate densities of striae on the valve face, or ‘small’, such as ultrastructural features seen only with electron microscopy. In fact, the new *Rhoicosphenia* species from CA were described based on differences in morphology, mainly valve shape (Thomas & Kociolek 2015), which is consistent with the differences found in other recently described *Rhoicosphenia* from other regions (Levkov et al. 2010, Nakov & Levkov 2008).

Because of the widespread use of a morphological species concept in diatoms, other aspects of diversity between populations, such as ecology, biogeography, and genetic distance, are not often discussed but could add information to species delimitation in diatoms (de Queiroz 2007). The recently described diversity from California and Oregon (Thomas et al. 2015) prompted investigations of *Rhoicosphenia* from streams throughout the US. Several unique morphologies were found across the US from sampling locations spanning ecological gradients (e.g. conductivity, pH, and various nutrients). Some studies have investigated the link between ecology and biogeography at continental scales (Bennett et al. 2010, Vyverman et al. 2007, Verleyen et al. 2009), and the relationship between species within a genus (of *Achnanthidium*) and ecology has also been addressed (Potapova & Hamilton 2007). However, this study aims to elucidate biogeographical patterns in the diatom genus *Rhoicosphenia* and determine whether or not these patterns can be explained by the water chemistry of the habitats in which they live. The goal of this study is not to make predictive statements, based on water chemistry data, as to which *Rhoicosphenia* may be found in any given location, but rather to provide concrete descriptive information of the niches that these eight taxa actually occupy. Ultimately the goal of this study is to demonstrate the utility of ecology and biogeography in delimiting species of *Rhoicosphenia*. Further investigations into the species diversity of *Rhoicosphenia* may be able to provide a more predictive assessment of this genus as it pertains to water quality conditions.

Materials and methods

Sample selection

For this study, a search through the ANS sample database was performed to identify sites where *Rhoicosphenia* exceeded 10% relative abundance. At abundances $\leq$ 10% it is difficult to positively identify *Rhoicosphenia* at the species level. From the total 4400 records, 749 sites were identified based on this criteria, and within these sites, all *Rhoicosphenia* species were previously identified as *R. abbreviata* (ANS et al. 2011–2016). Of the 749 samples, there were 501 samples from across the US from studies by the United States Geological Survey (USGS) National Water-Quality Assessment Program (NAWQA). The other major source of data was from the state of California, with a total of 248 samples. Of the CA samples, 229 were part of the Surface Water Ambient Monitoring Program (SWAMP) throughout the state, and the other 19 came from a study that concentrated on coastal watersheds in the Southern California Bight (SCB) from Santa Barbara in the North, San Diego in the South, and San Bernardino in the East. A detailed list of all samples examined including project, sample ID, latitude, longitude, and taxon can be found in Appendix A as well as at figshare.com (DOI 10.6084/m9.figshare.3115363).

Sites with ecological data

Of the 749 sites identified with sufficient *Rhoicosphenia* populations, 536 were used in the ecological analysis. Sites were removed if they lacked sufficient water quality data for statistical analyses or if the algal sample and water quality sample were collected more than one month apart. If the samples were collected more than one month apart, the taxon found may not be accurately representative of conditions across that temporal span due to the rapid response of algal communities to environmental change. Of the 536 samples analyzed, there were 182 from California (n = 164 SWAMP, n = 18 SCB) and 354 from US sources. A detailed list of all samples examined including project, sample ID, latitude, longitude, taxon, and water chemistry parameters are included for the samples used in statistical analyses can be found in Appendix A as well as at figshare.com (DOI 10.6084/m9.figshare.3115363).

Taxon identifications

Prior to this dissertation, only one freshwater *Rhoicosphenia* species was commonly reported from the US, *R. abbreviata*, although its synonym *R. curvata* has also been widely reported (mostly prior to 1980). During the observations of the samples from across the US analyzed in this study, *R. abbreviata* as defined by the type material was not identified in any sample, but eight other taxa were found. The taxa identified and used in these analyses are three described species (Thomas & Kociolek 2015), and several morphotypes that have not yet been described (see Chapter 2 of dissertation). The three described species are *Rhoicosphenia californica* E.W. Thomas & Kociolek, *R. lowei* E.W. Thomas & Kociolek, and *R. stoermeri* E.W. Thomas & Kociolek, and the five currently unpublished morphotypes, which are designated as *Rhoicosphenia sp. 1*, *R. sp. 2*, *R. sp. 3*, *R. sp. 4*, and *R. sp. 5*, are used in these analyses. Descriptions and images of each species and morphotype can be found in Chapter 2 of this dissertation. In terms of frequency of occurrences in the data examined, *R. californica* was found in 215 (153) sites, *R. lowei* in 118 (65), *R. stoermeri* in 15 (13) (least common), *R. sp. 1* in 261 (193) (most common), *R. sp. 2* in 76 (54), *R. sp. 3* in 127 (89), *R. sp. 4* in 16 (15), and *R. sp. 5* in 57 (50); the first number is the total number of samples with the taxon, the number in parenthesis is the number of samples for that taxon with ecological data. Of the 750 total sites, 130 sites had more than one taxon present (approximately 17.3% of sites). Based on the examinations of stream and river samples across the US, these species represent the currently known diversity of *Rhoicosphenia*. Based on the descriptions and images of the eight taxa, a matrix for comparison of overall morphological similarity is presented in Table 1.

Biogeography of Rhoicosphenia taxa

Latitude and longitude data from 750 sampling locations with *Rhoicosphenia* taxa present were used to generate a map of occurrence throughout the US. In addition to the 'static' map (Figure 1), the site data from Appendix A was transformed into a ".kmz" file for dynamic viewing in Google Earth and this ".kmz" file is available for download from figshare.com, (DOI 10.6084/m9.figshare.3115369). To determine the range overlap of the taxa, the location information for species pairs was compared and represented as a percentage of shared locations. For example, if species A was found in 50 sites, species B was found in 150 sites, and they co-occur in 20 sites, their range overlap would be 10% (=20/(50+150)). These values can be found in Table 2. This method was chosen over comparing species ranges based on polygons of biogeographical range because it examines the actual, rather than inferred, ranges.

Statistical analyses and data analyzed for niche comparison

Non-Metric Multidimensional Scaling (NMDS) of water chemistry and taxon distribution among sites was performed to visualize ecological distances between sampling locations. The water quality parameters for this analysis do not meet the assumption of normality, therefore

NMDS was used as it is more appropriately suited to non-parametric data, whereas an ordination such as Principal Components Analysis (PCA) would be an analogous test if the data were normally distributed. Samples used in this study were collected between 1993 and 2010, and over that time not all water chemistry variables were measured at each site. This non-standardized sampling regime could have been done for practical purposes in terms of what analyses were deemed to be more or less important over time and between studies, but the end result is that not all sites have the same set of data available for analysis. In order to maximize the number of water chemistry variables and reduce the amount of missing data to zero, the variables included were pH, phosphorus (mg/L), silica (SiO_2, mg/L), specific conductivity (µS/cm), and sulfate (mg/L). The variables phosphorus, silica, specific conductivity, and sulfate were log transformed due to orders of magnitude variability of those parameters (Pan et al. 1996); pH was not transformed as it is already measured in a log scale. The statistical program R version 3.2.3 (R Core Team 2015) was used to build a dissimilarity matrix on Bray-Curtis similarities, and then perform NMDS among water quality parameters and algal sampling locations based on the similarity matrix.

Since NMDS is a graphical display of data points, and not a statistical test, it was followed by the non-parametric statistical test Analysis of Similarity (ANOSIM), a tool for permutation-based hypothesis testing, was performed to determine significance between pairwise comparisons of the taxa. ANOSIM was performed in PRIMER 5.2.9 (Clarke 2001). The results of ANOSIM are pairwise comparisons of taxa, 28 in total, and indicate whether or not the taxa compared share niche space. The R statistic for each pair is reported in Table 3, and R statistic values above 0.2 are considered to show a strong relationship. A Bonferroni correction was applied and reduced the alpha level (initially set at 0.05) to account for the multiple pairwise comparisons making the new alpha level for significance of ANOSIM results 0.002.

PRIMER was also used to run a Similarity percentages analysis (SIMPER) to identify variables contributing to differences identified between species pairs identified as having significantly different niches. SIMPER analysis ranks the contribution of variables to the differences in pairs of taxa and allows for a better understanding of how specific variables in a multivariate analysis, such as NMDS, effect specific species pairs in somewhat univariate way.

Box plots were created in Past 3.07 (Hammer 2015) as a way to visualize the univariate niche space of the taxa based on each water quality variable. Each of the five variables (pH, phosphorus, silica, specific conductivity, and sulfate) have their own set of box plots, with one box for each of the eight species. These box plots are made with the untransformed data and are thus presented as individual figures due to the differences in scale for the measures variables.

A Mantel's test on the association between geographical distance and similarity of ecological preferences was computed for each taxon. The question the Mantel's test addresses is whether or not geographical distance is correlated with niche. For each taxon, two matrices were computed to use to generate the Mantel statistic. First, a similarity matrix based on the Bray-Curtis distances between ecological parameters was computed; second, the Haversine straight line distances (in meters) between sampling locations with a particular taxon were computed and used to generate the other similarity matrix. The ecological data were log transformed (with the exception of pH). The two similarity matrices were plotted with geographic distance (m) on the x-axis and ecological similarity (presented as Bray-Curtis dissimilarity) on the y-axis. For Bray-Curtis dissimilarity, a value of 0 indicates that two locations are most similar (i.e., the same) and a value of 1 indicates that two locations are most different (i.e., not at all the same). All analyses were run in R with the “sp”, “geosphere”, “permute”, and “vegan” packages.

Results

Taxon comparisons

Taxon	*R. stoermeri*	*R. lowei*	*R. californica*	*R. sp. 1*	*R. sp. 2*	*R. sp. 3*	*R. sp. 4*
R. sp. 5		Similar				Similar	
R. sp. 4							
R. sp. 3		Similar					
R. sp. 2			Similar	Similar			
R. sp. 1			Similar				
R. californica							
R. lowei	Similar						

Table 1: Comparison of species based on morphology. “Similar” means that based on morphology they share some characteristics, if taxa are very easily distinguishable, nothing is written.

The morphological distinctions between these taxa are discussed in detail in the species descriptions of Chapter 2 of this dissertation. In the broader context of diatom species delimitation with the morphological species concept, all of these taxa have sufficient distinctions to merit their recognition as separate morphological species. The three taxa from California, *R. californica*, *R. lowei*, and *R. stoermeri* have been accepted though peer review, however, the five unnamed taxa (*R. sp. 1*, *R. sp. 2*, *R. sp. 3*, *R. sp. 4*, and *R. sp. 5*) have not yet been submitted for review.

Biogeographical patterns

Rhoicosphenia taxa were observed in samples from 38 of the 48 contiguous states due to the availability of samples with associated water quality data. Results of mapping the distributions of *Rhoicosphenia* in the US show that some taxa overlap in their geographical ranges with each other, while others do not (Figure 1). *Rhoicosphenia sp. 3* is the most widespread, with a range extending from the Mountain West to the Appalachian Mountains in the East. *Rhoicosphenia sp. 1* is also widespread, most commonly found West of the Mississippi River, but also in some sites in the Mountain West. *Rhoicosphenia sp. 2* is common in the Mountain West, Pacific Northwest, and Northern Plains, while *R. sp. 4* is restricted to New Mexico and Arizona. *Rhoicosphenia californica* is very common in California, but also occurs in southern NM and Oregon, while *R. stoermeri* is restricted to CA. *Rhoicosphenia sp. 5* is most common in southern CA, but is also seen in Nevada and generally in CA. *Rhoicosphenia lowei* is common from CA in the west to the Rocky Mountains in the east, but does not occur in the plains east of the Rockies. California is home to the greatest number of species, with five of eight taxa being found within the state. The results of the mapping indicates that not all species and morphotypes are evenly distributed across the country, and that while some ranges overlap and taxa co-occur, they have relatively well defined distributions. Co-occurrence of taxa was found in ~17.3% of sites, and biogeographical overlap as a percentage of common sites between species pair is found in Table 2.

Taxon	*R. stoermeri*	*R. lowei*	*R. californica*	*R. sp. 1*	*R. sp. 2*	*R. sp. 3*	*R. sp. 4*
R. sp. 5	0 (0)	0.5 (1)	5.5 (15)	0 (0)	0 (0)	0 (0)	0 (0)
R. sp. 4	0 (0)	0 (0)	0 (0)	0 (0)	2.2 (2)	0 (0)	
R. sp. 3	0 (0)	5.3 (13)	0 (1)	6.9(27)	7.4(15)		
R. sp. 2	0 (0)	2.5 (5)	0 (0)	0 (0)			
R. sp. 1	0 (0)	0 (1)	0 (0)				
R. californica	3.5 (8)	8.4 (33)					
R. lowei	0 (0)						

Table 2: Percentage of sites shared by species pairs. Number of shared sites in parentheses.

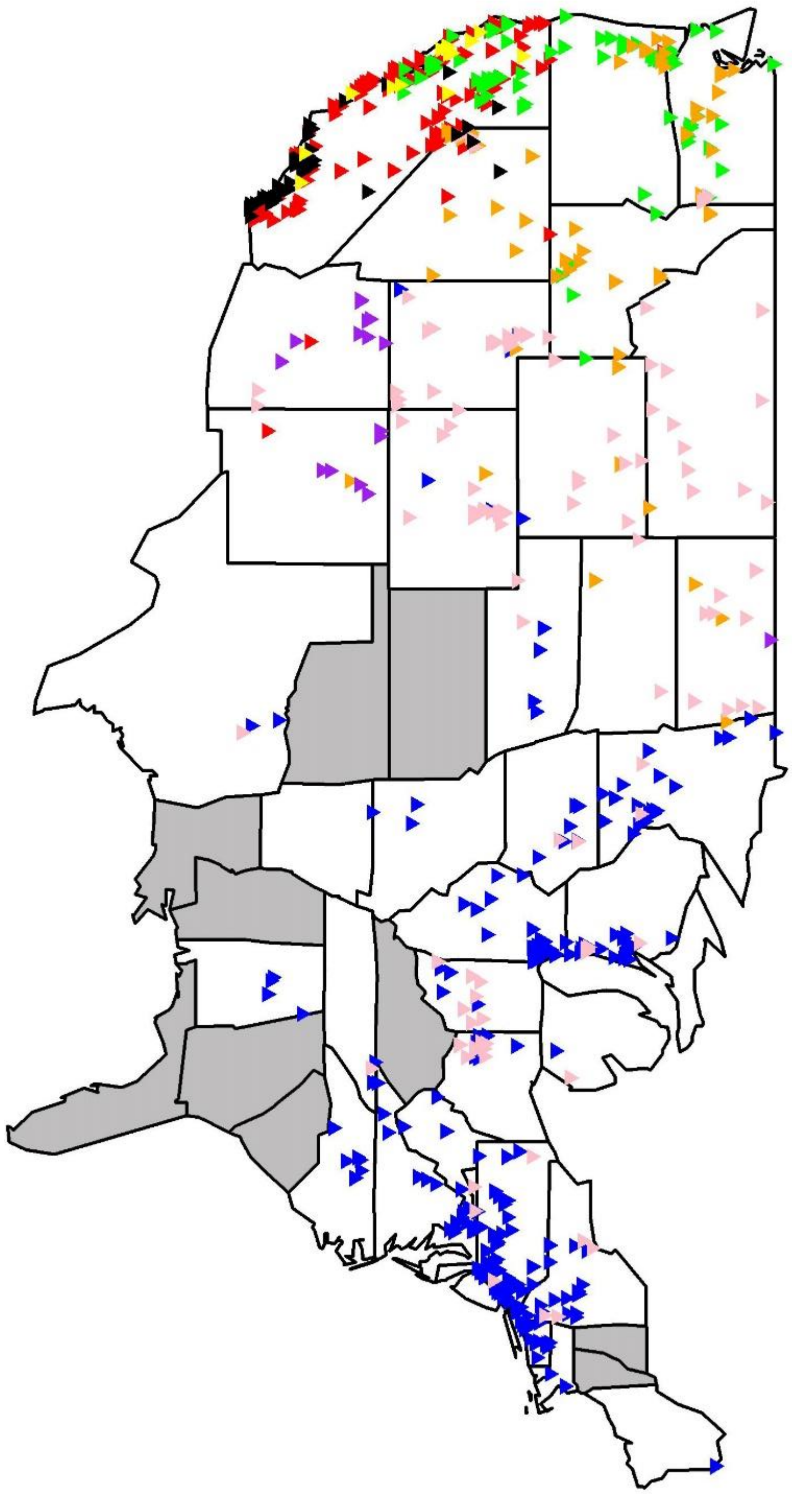

Figure 1: Map of United States with known locations of the eight *Rhoicosphenia* taxa. *R. californica* (red), *R. lowei* (green), *R. stoermeri* (yellow), *R. sp. 1* (blue), *R. sp. 2* (orange), *R. sp. 3* (pink), *R. sp. 4* (purple), *R. sp. 5* (black). Locations represent collections from 38 of the 48 contiguous United States, states shaded in gray had no collections in database to examine.

Niche comparison

NMDS

Results show that taxa are distributed across ecological space (as represented in the two-dimensional NMDS plot, stress=0.16), and that most species are clustered closer together with each other than with other taxa. While *R. sp. 1* is common, its NMDS distribution is relatively compact near the center of the plot, this is also true for *R. sp. 5*, however it does not overlap with the distribution of *R. sp. 1*. *R. californica* has occupies the broadest space in the plot, while *R. sp. 4* occupies the narrowest space.

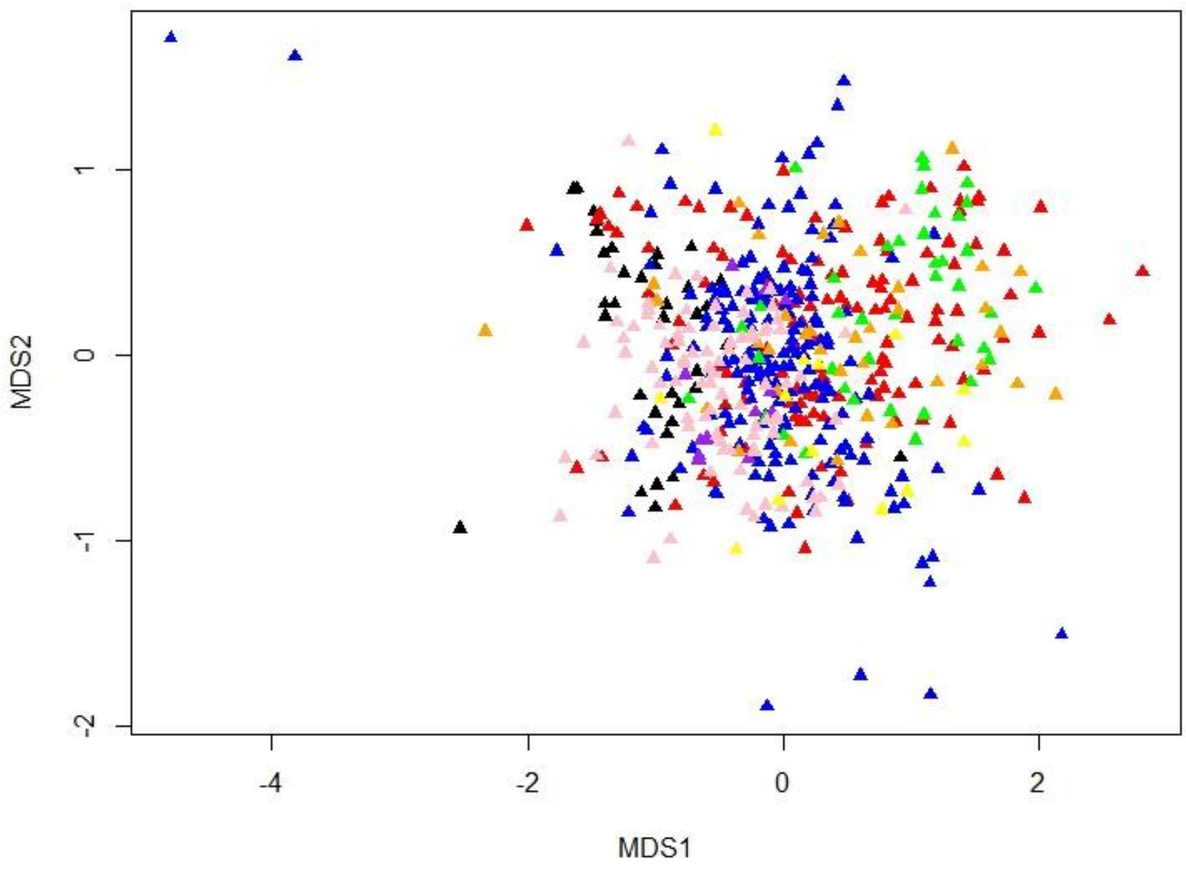

Figure 2: NMDS plot with all taxa represented by different colored symbols (*R. californica* (red), *R. lowei* (green), *R. stoermeri* (yellow), *R. sp. 1* (blue), *R. sp. 2* (orange), *R. sp. 3* (pink), *R. sp. 4* (purple), *R. sp. 5* (black)).

The NMDS plot was also used to illustrate difference in the ecological niche between species pairs. Fig. 3 focuses on 2 species, *R. sp. 2* (yellow triangles) and *R. lowei* (green triangles) highlighted with colored symbols, with the remaining taxa represented by gray symbols. *Rhoicosphenia sp. 2* and *R. lowei* are a pair of species that share niche space, as determine by ANOSIM results (Table 3). Figure 3 demonstrates that while the spatial arrangements of sampling locations for these taxa, as displayed in multidimensional space, have a high degree of overlap and they share overall niche space. In regards to their biogeographical distributions, they are found to co-occur in 2.5% of samples and are both most common in the Western US. In terms of morphology, they have very distinct valve shapes.

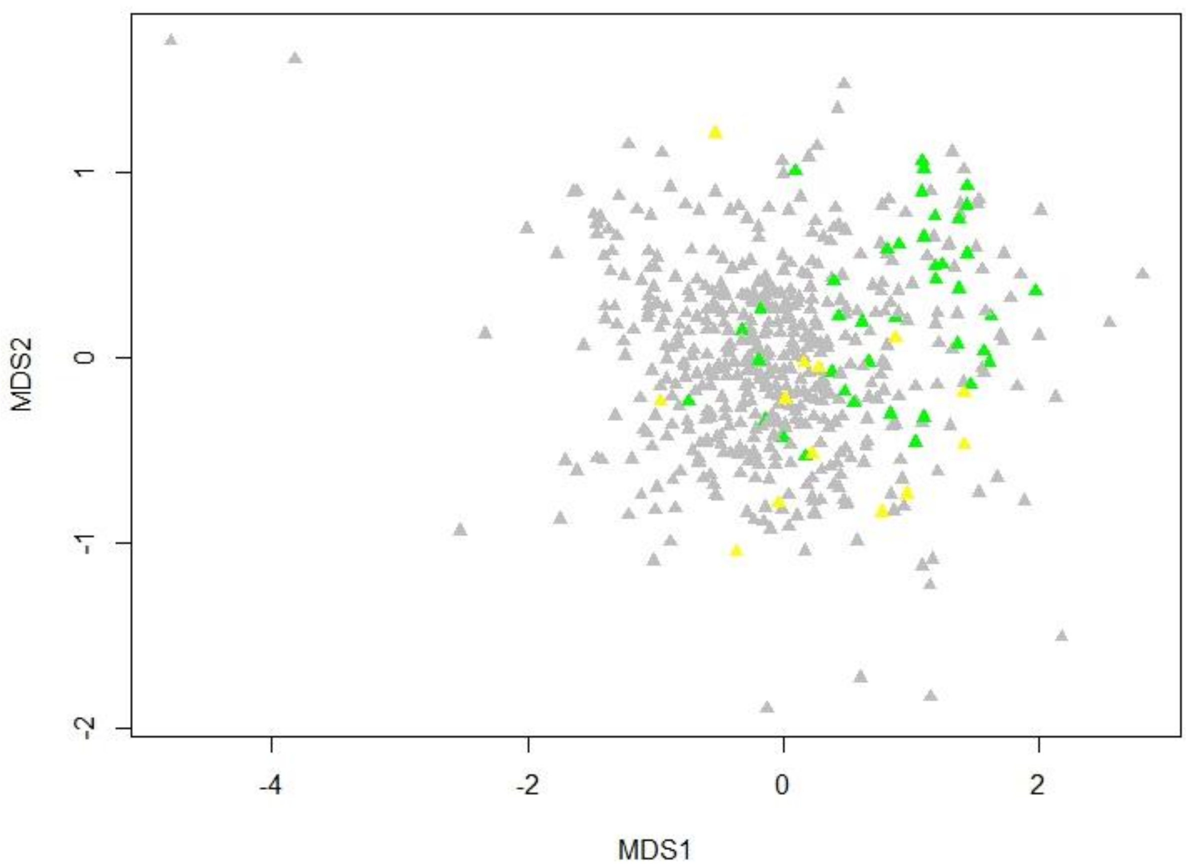

Figure 3: NMDS plot with all taxa represented by different gray symbols, except *R. lowei* (green) and *R. stoermeri* (yellow), which occupy the same niche space.

Figure 4 focuses on 2 species, *R. californica* (red triangles) and *R. sp. 1* (blue triangles), highlighted with colored symbols, with the remaining taxa represented by gray symbols. *Rhoicosphenia californica* and *R. sp. 1* are a pair of taxa that have statistically different niche space, as determine by ANOSIM results (Table 3). Even though there is a minor degree of overlap of the points for these taxa, there is a statistical difference in their realized niches. These taxa have no common sites, *R. californica* is most common in California and some neighboring states, while *R. sp. 1* is most common east of the Rocky Mountains. However, they are two of the more morphologically similar taxa based on their descriptions and images.

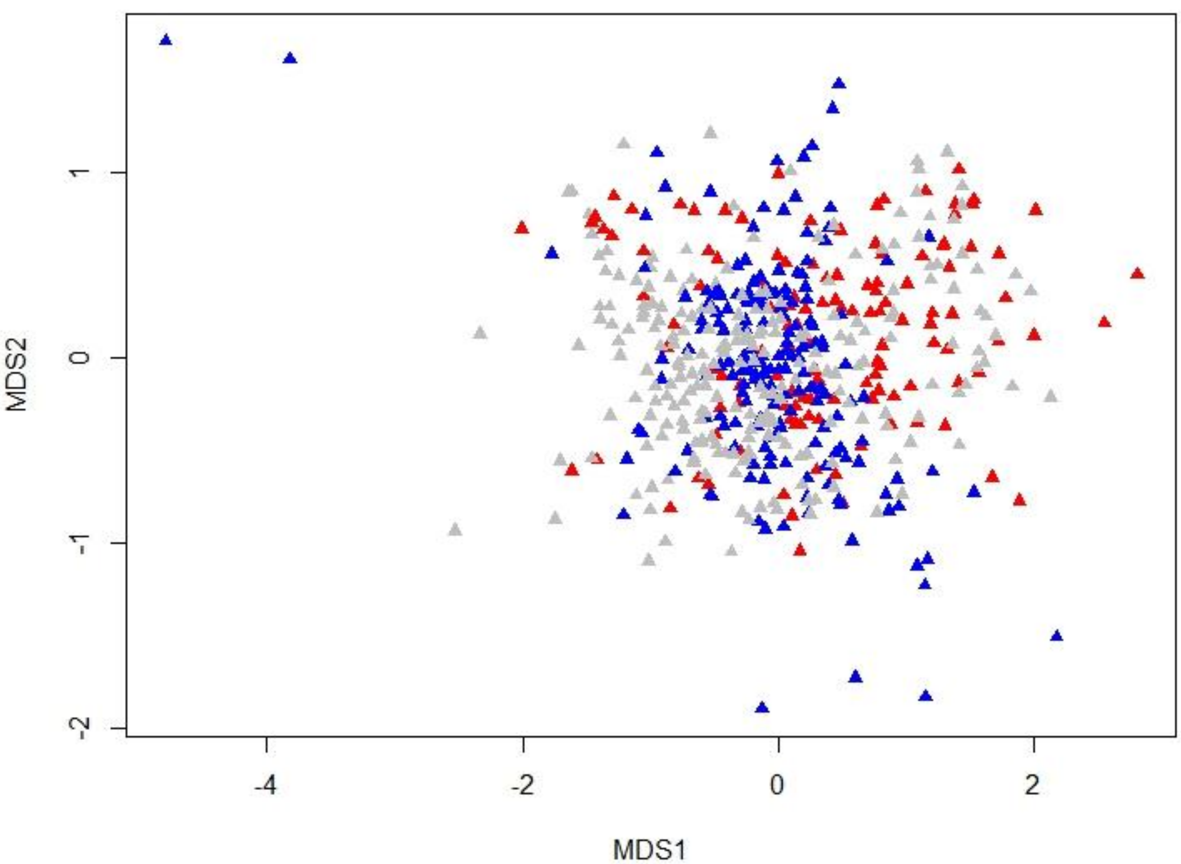

Figure 4: NMDS plot with all taxa represented by different gray symbols, except *R. californica* (red) and *R. sp. 1* (blue), which occupy statistically different niche space.

<u>ANOSIM</u>

The results of the ANOSIM analysis produced a Global R = 0.243 and p=0.001, after 999 permutations. The alpha level of 0.05 was adjusted to 0.002 for Bonferroni correction to account for the 28 pairwise comparisons. The parameter estimate for ANOSIM is an R statistic, with values closer to 0 indicating no difference exists between pairs, values closer to 1 indicating the pairs are different. ANOSIM values greater than 0.2 are considered strong predictors of a correlation between a taxon and the measured variables. Of the 28 pairs, 12 taxa could not be distinguished based on niche alone (R statistic < 0.200, p > 0.002), while the other 16 pairs had R statistic values >0.200 and significant p-values, allowing for their distinction based on niche requirements. R statistic values in black text represent a correlative relationship, and stars (*) represent a statistically different niche as calculated by ANOSIM. Fields containing red text represent taxa that do not have statistically different niches.

Taxon	*R. stoermeri*	*R. lowei*	*R. californica*	*R. sp. 1*	*R. sp. 2*	*R. sp. 3*	*R. sp. 4*
R. sp. 5	0.503*	0.524*	0.361*	0.472*	0.292*	0.264*	0.119
R. sp. 4	0.309*	0.149	0.091	0.079	-0.071	-0.090	
R. sp. 3	0.389*	0.363*	0.275*	0.135*	0.245*		
R. sp. 2	-0.024	0.052	0.066	0.332*			
R. sp. 1	0.320*	0.308*	0.279*				
R. californica	0.027	-0.028					
R. lowei	0.045						

Table 3: Results of ANOSIM on ecological variables. The parameter estimate for ANOSIM is an R statistic, closer to 0 means no difference, closer to 1 means different, with values greater than 0.2 being considered strong predictors. P-values are also reported, and when p-values were less than or equal to 0.002, an asterisk (*) is placed next to the R statistic value. Species pairs with low (R<0.200) statistical relationships (fail to reject the null hypothesis) are written in red text.

When only *R. abbreviata* was reported from the US, its niche was demonstrated to be very broad. The recognition of more taxa has decreased the niche space for the taxa, and the results of the ANOSIM demonstrate that not all of their niches overlap – 16 of the 28 taxon pairs show statistical difference in their niche, while the other 12 pairs have overlapping niches. These results are most compelling in that the adoption of these new taxa into monitoring studies can add predictive information about site conditions based on which taxon is found.

SIMPER

The SIMPER analysis was used to determine which ecological variables drive the differences found between taxa in the ANOSIM analysis. For all 28 pairwise comparison, sulfate contributed the most to the differences in niche between taxa with the minimum difference for sulfate between taxa being 41.15% found between *R. sp. 4* (avg. 106.9 mg/L) & *R. sp. 5* (avg. 460.5 mg/L). The greatest difference for sulfate (59.86%) was identified between *R. stoermeri* (avg. 50.0 mg/L) & *R. sp. 4* (avg. 106.9 mg/L). Full SIMPER results are available in Table 4 which includes the taxon pair, and the variables in order of highest to lowest effect on differences. Taxa that were shown to have statistically different niches in the ANOSIM results are in **bold** font in the table. The average values for each of the niche parameters can be found in Table 5, which can be helpful to observe the differences between parameter values for the taxon pairs analyzed with SIMPER. SIMPER results show that sulfate contributed the largest proportion of distinction between niches of the two compared taxa, whether they were statistically significant based on ANOSIM results or not. Conductivity and silica also contributed a large proportion of the distinction between taxa. pH and phosphorus contributed the least to the differences between taxa.

Taxon Pair	Variable				
	S	**SC**	**Si**	**P**	**pH**
R. californica* & *R. sp. 3	46.81	26.51	24.01	1.55	1.11
R. californica* & *R. sp. 5	54.14	31.41	12.27	1.25	0.93
R. lowei* & *R. sp. 3	48.86	25.76	23.02	1.46	0.90
R. lowei* & *R. sp. 5	55.73	30.24	12.09	1.12	0.82
R. stoermeri* & *R. sp. 1	46.04	25.58	23.02	3.20	2.15
R. stoermeri* & *R. sp. 3	53.00	22.83	21.11	1.88	1.18
R. stoermeri* & *R. sp. 4	59.86	24.52	13.00	1.44	1.18
R. stoermeri* & *R. sp. 5	58.78	27.43	11.24	1.38	1.16
R. sp. 1* & *R. sp. 2	43.54	27.55	24.32	3.07	1.52
R. sp. 1* & *R. sp. 3	45.80	24.99	23.46	3.87	1.88
R. sp. 1* & *R. sp. 5	48.38	27.70	20.37	2.44	1.11
R. sp. 2* & *R. sp. 3	46.93	26.87	23.20	2.04	0.96
R. sp. 2* & *R. sp. 5	51.71	31.10	14.57	1.61	1.01
R. californica & *R. sp. 2*	49.96	29.12	17.77	1.79	1.36
R. californica & *R. sp. 4*	51.85	27.47	18.19	1.41	1.09
R. lowei & *R. sp. 2*	49.98	28.60	18.53	1.72	1.17
R. lowei & *R. sp. 4*	54.38	26.35	17.13	1.29	0.84
R. stoermeri & *R. sp. 2*	51.81	29.36	15.50	1.19	1.42
R. sp. 1 & *R. sp. 4*	47.16	26.43	20.70	3.87	1.83
R. sp. 2 & *R. sp. 4*	50.69	28.71	17.71	1.98	0.91
R. sp. 4 & *R. sp. 5*	41.15	33.16	21.92	2.37	1.40
	S	**Si**	**SC**	**P**	**pH**
R. californica* & *R. sp. 1	41.73	27.62	26.68	2.58	1.39
R. lowei* & *R. sp. 1	42.56	27.10	26.34	2.59	1.41
R. sp. 3* & *R. sp. 5	44.18	26.94	25.29	2.24	1.34
R. sp. 3 & *R. sp. 4*	41.76	27.55	26.34	3.17	1.17
	S	**SC**	**Si**	**pH**	**P**
R. californica & *R. lowei*	49.43	29.36	18.32	1.49	1.40
R. californica & *R. stoermeri*	49.86	30.55	16.33	1.82	1.44
R. lowei & *R. stoermeri*	49.18	30.7	17.13	1.64	1.35

Table 4: SIMPER results showing the three orders of contribution to difference found in the analysis. For each taxon pair, the variables are placed in descending order from highest, to lowest contribution to percentage difference. Taxa that were shown to have statistically different niches based on results of ANOSIM are in **bold** font.

Taxon	**Variable**				
	S	**SC**	**Si**	**P**	**pH**
R. californica	82.64	475.39	26.77	0.06	7.97
R. lowei	22.76	325.42	24.49	0.05	8.12
R. stoermeri	50.04	554.44	16.54	0.03	8.28
R. sp. 1	50.28	476.68	9.02	0.22	7.85
R. sp. 2	117.11	568.81	20.74	0.09	8.22
R. sp. 3	151.61	772.24	26.2	0.10	8.27
R. sp. 4	103.44	769.81	12.64	0.05	8.25
R. sp. 5	480.48	1737.95	21.57	0.09	7.97

Table 5: Average values of ecological variables for the eight taxa in this study. These values are not log transformed. (S = Sulfate, SC = Specific Conductivity, Si = Silica, P = Phosphorus)

Box Plots

pH values in this study range from 5.8–9.74. Box plot analysis reveals that while some taxa (*R. californica*, *R. sp. 1*, and *R. sp. 5*) are found mostly in ranges of pH from ~7.0–9.0 (*R. sp. 1* down to 5.8), their interquartile ranges are narrow (<1 pH point). This means that the majority of the within taxon variation in regards to pH is not that great and pH may be predictive of *Rhoicosphenia* taxa present in given conditions. However, there is little variability in median values (black vertical lines in boxes) for several taxa such as *R. stoermeri*, *R. sp. 2*, and *R. sp. 3*.

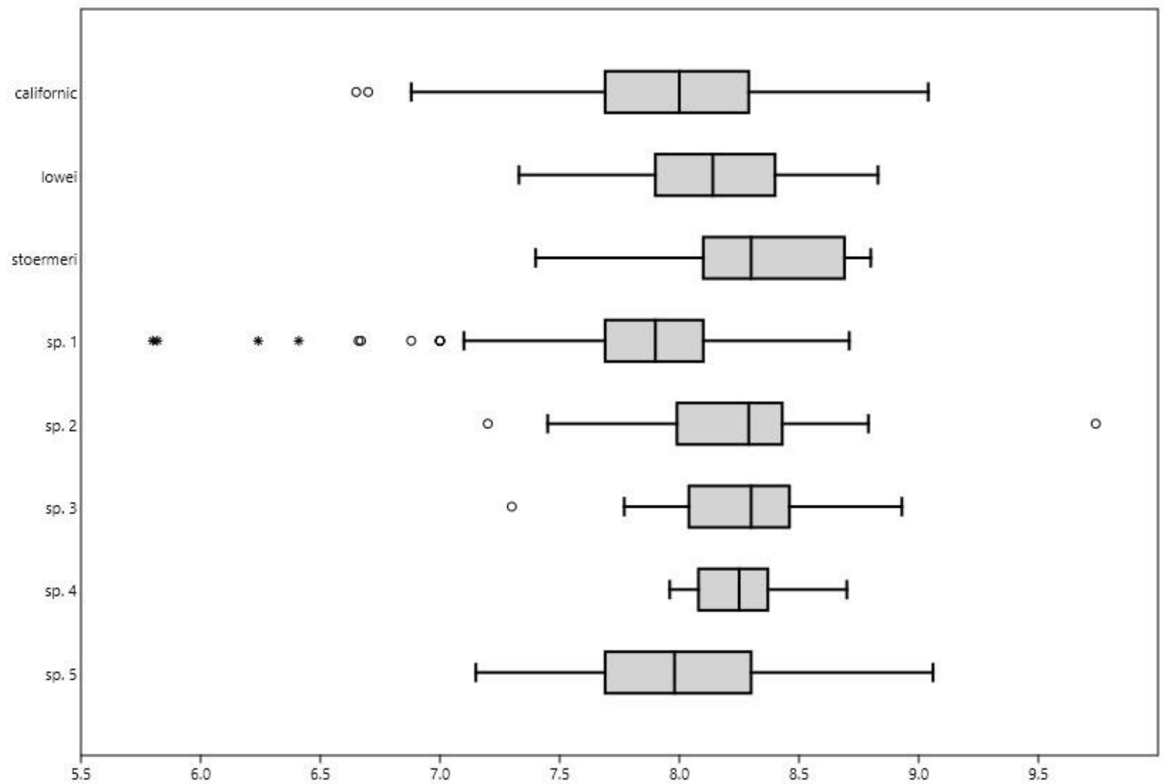

Figure 5: pH box plot. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error, with outliers represented by open circles and asterisks.

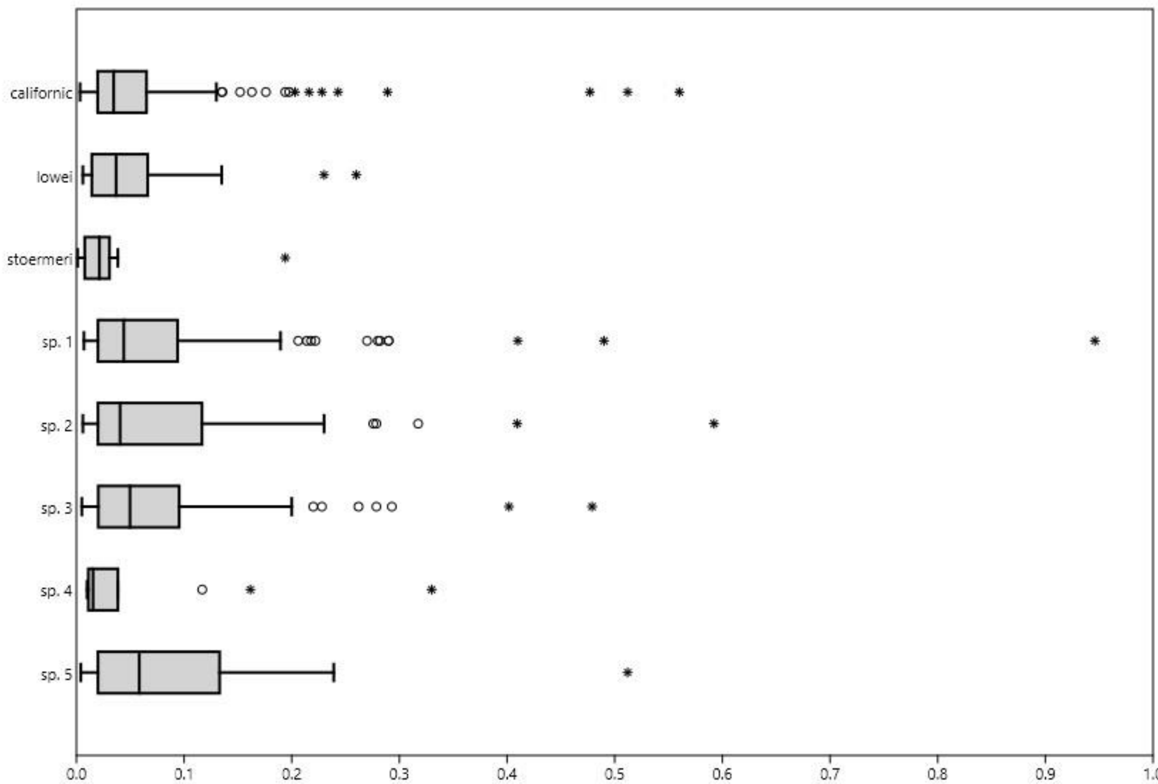

Figure 6: Phosphorus box plot. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error, with outliers represented by open circles and asterisks.

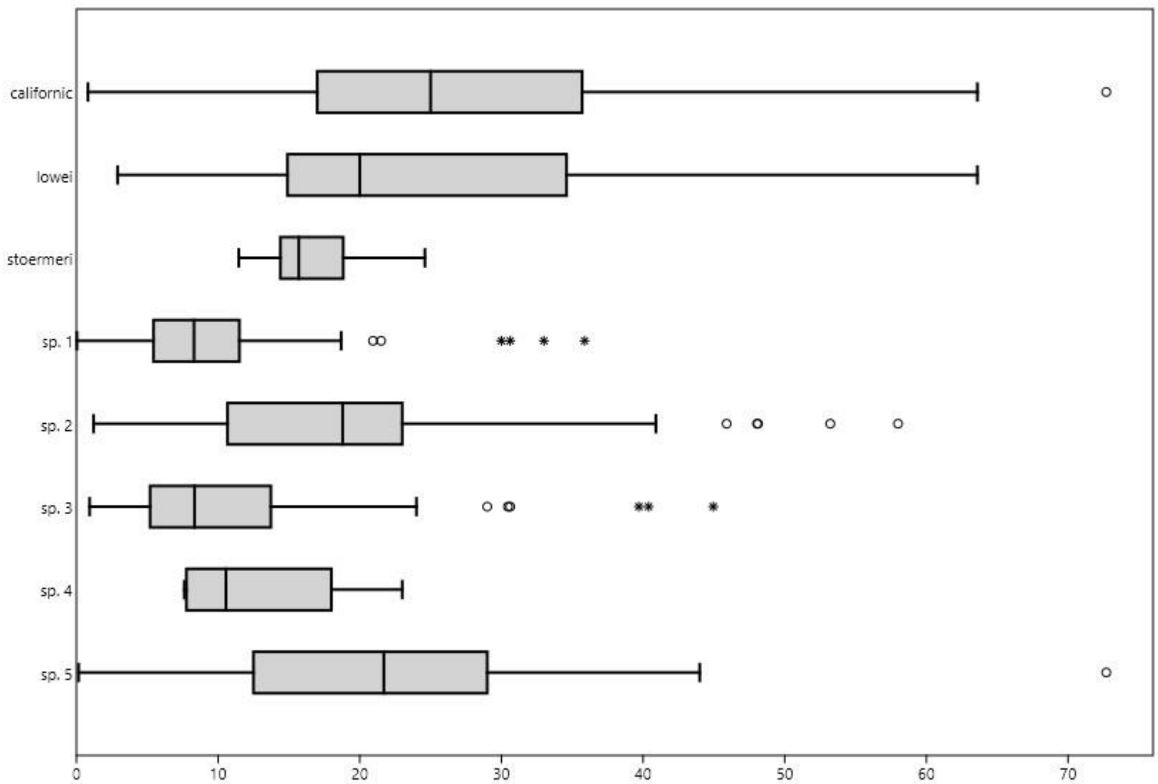

Figure 7: Silica box plot. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error, with outliers represented by open circles and asterisks.

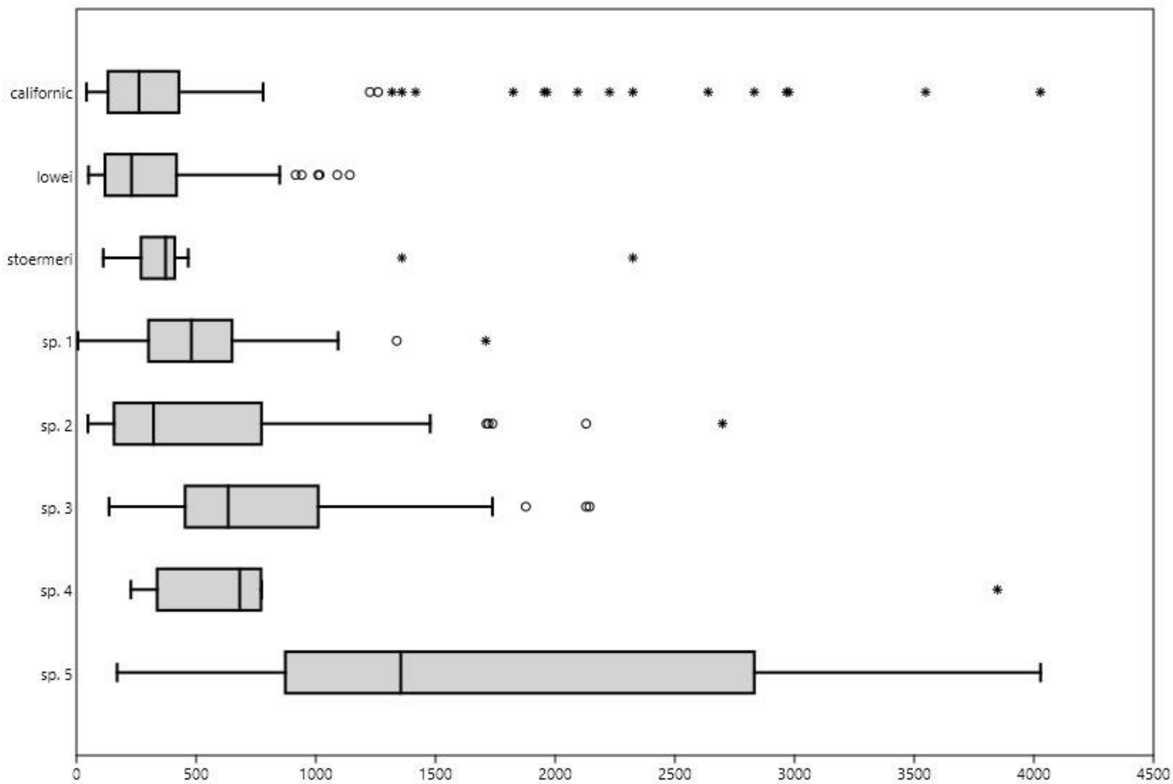

Figure 8: Specific conductivity box plot. Both boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error, with outliers represented by open circles and asterisks.

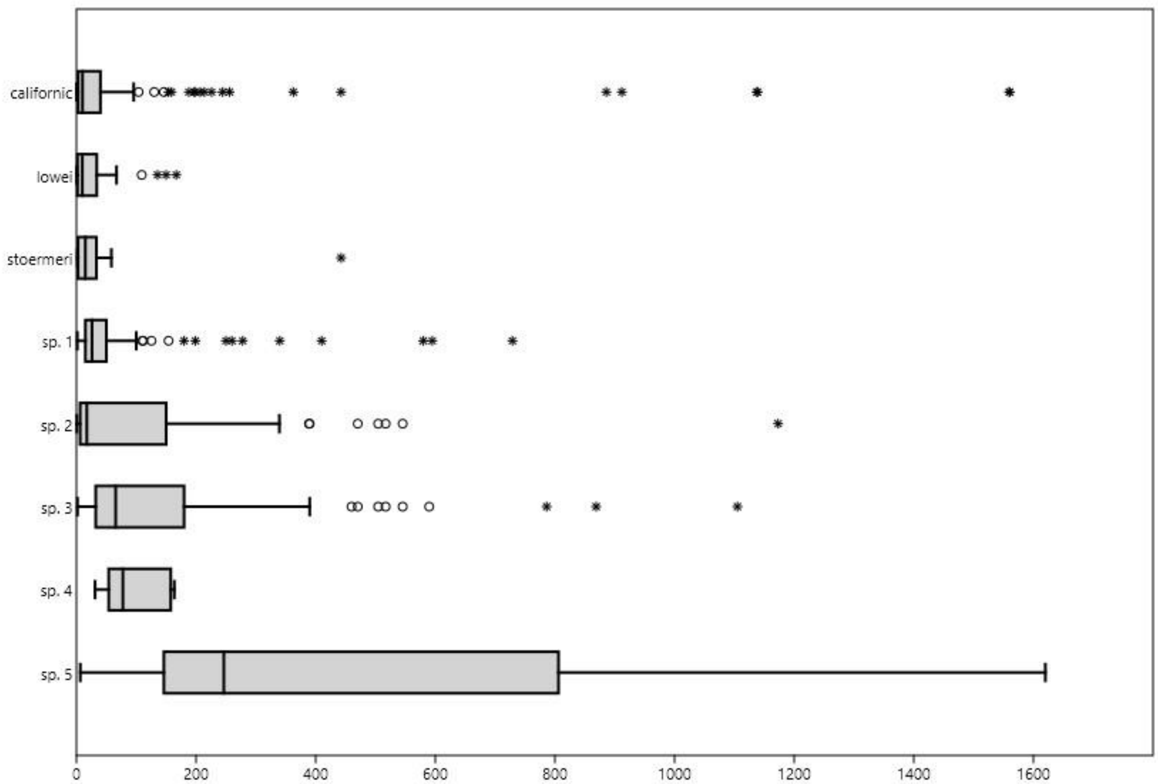

Figure 9: Sulfate box plot. Boxes representing interquartile range, vertical bar inside box is the median, and whiskers represent standard error, with outliers represented by open circles and asterisks.

The box plots are a way to visualize the differences in niche, one variable at a time. While multivariate techniques are preferred when many variables are available (as is the case in this chapter), box plots can still add to our understanding of the niches of these taxa. All of the box plots demonstrate that the range of variation of ecology that each species occupies is distinct amongst the other taxa. Further, the ranges of a taxon pair, for example *R. stoermeri* & *R. sp. 1*, may overlap for one variable (in this case sulfate), but other variables do not overlap (pH, phosphorus, silica). The box plots are also useful in discerning the variation in the range of values for certain variables.

Mantel's Tests

Mantel's tests were generated to understand the association between the ecological niches and geographic distances between locations where each taxon was observed. For all species except *R. sp. 1*, there was a statistically significant correlation between these two variables, with ecological similarity decreasing (i.e., more dissimilar) as geographic distance increased. The taxa *R. sp. 1* (Figure 13), *R. sp. 3* (Figure 15) have the largest geographic ranges and also have broad ecological ranges. *R. californica* (Figure 10), *R. lowei* (Figure 11), *R. sp. 2* (Figure 14), and *R. sp. 5* (Figure 17) are more regional in their distributions and also have moderate ecological range. *R. stoermeri* (Figure 12) and *R. sp. 4* (Figure 16) have both the smallest geographical and ecological ranges.

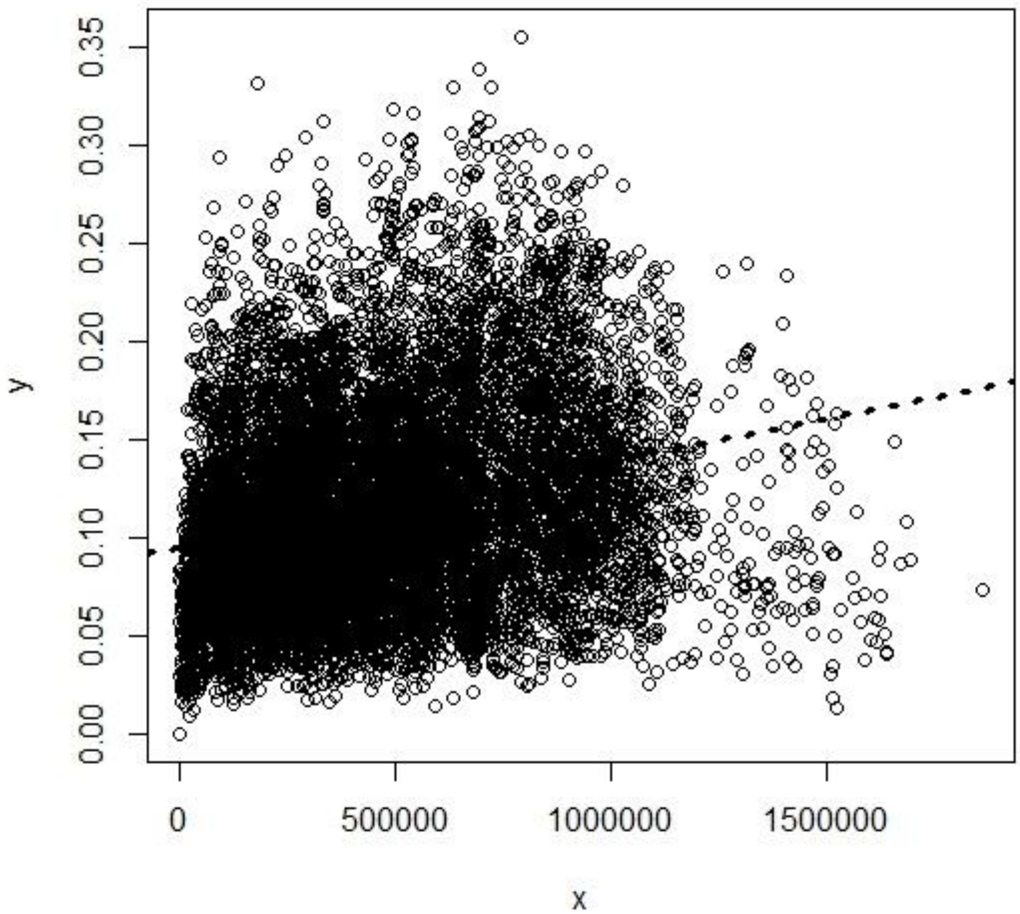

Figure 10: Mantel test *Rhoicosphenia californica*. Mantel statistic r: 0.2344, Significance: 0.001

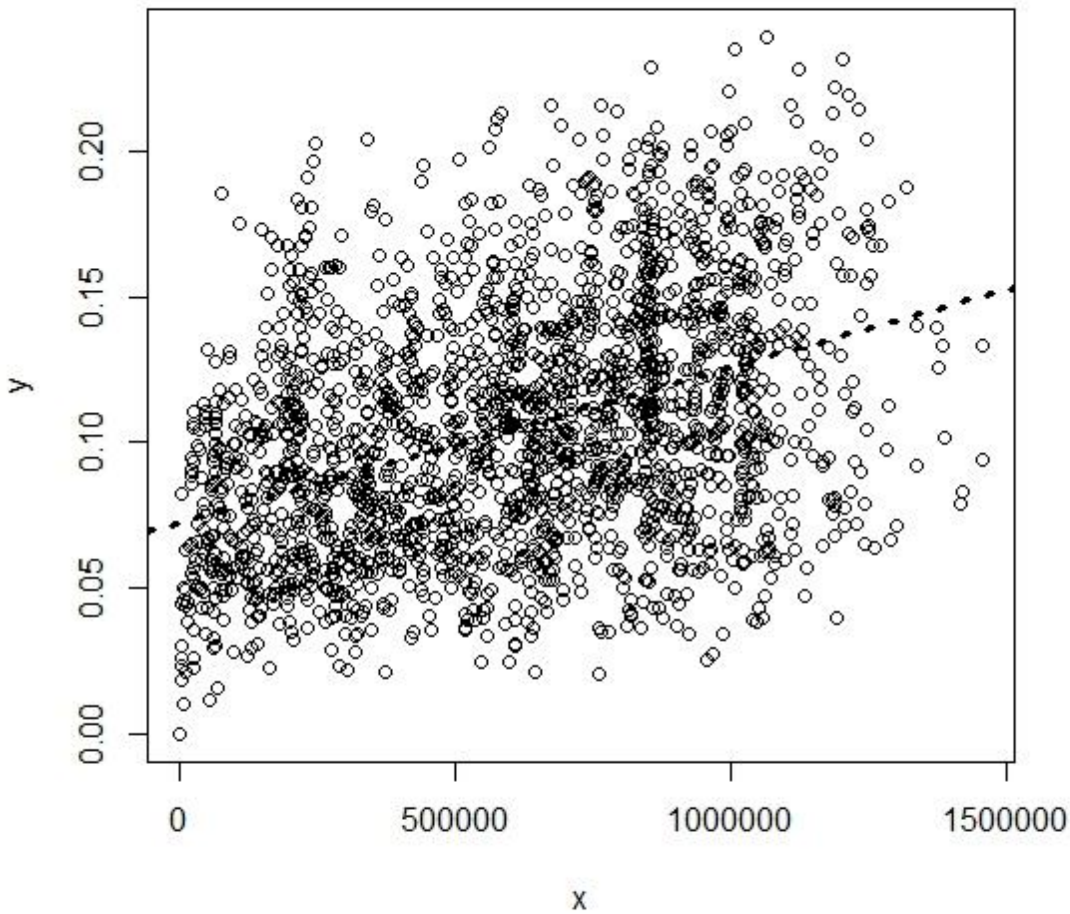

Figure 11: Mantel test *Rhoicosphenia lowei*. Mantel statistic r: 0.3598, Significance: 0.001

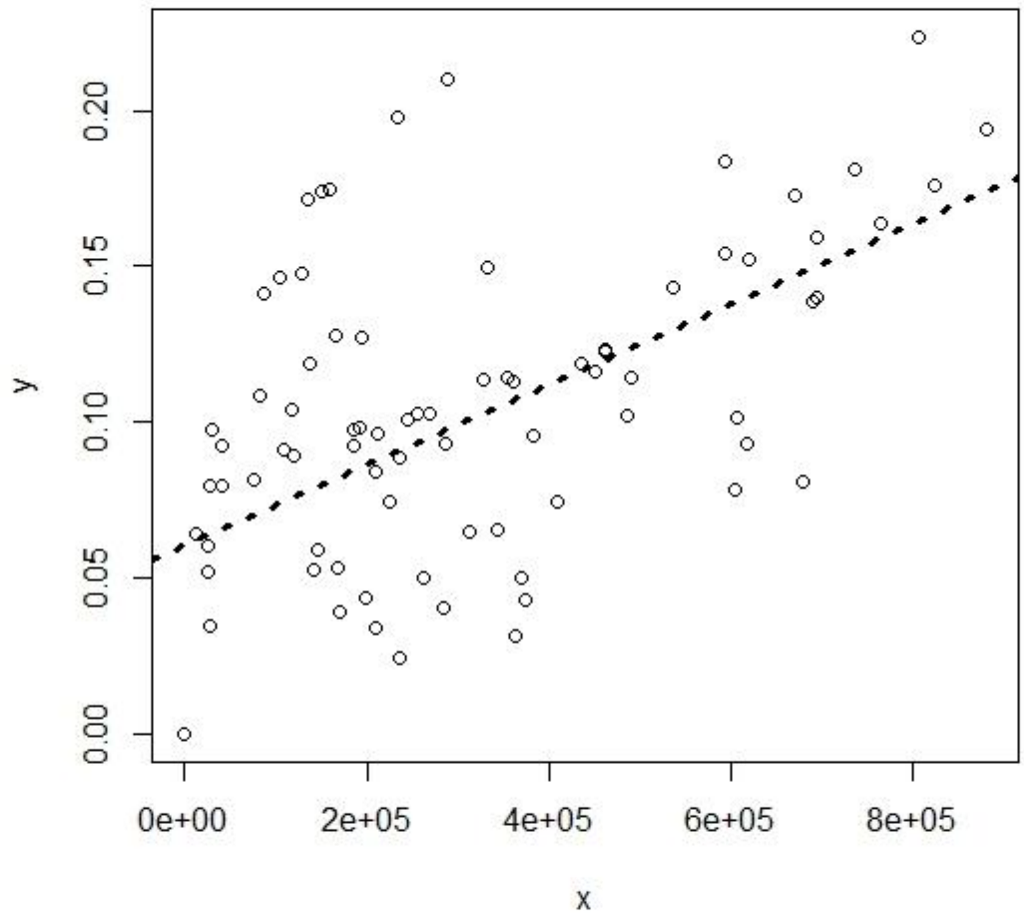

Figure 12: Mantel test *Rhoicosphenia stoermeri*. Mantel statistic r: 0.475, Significance: 0.008

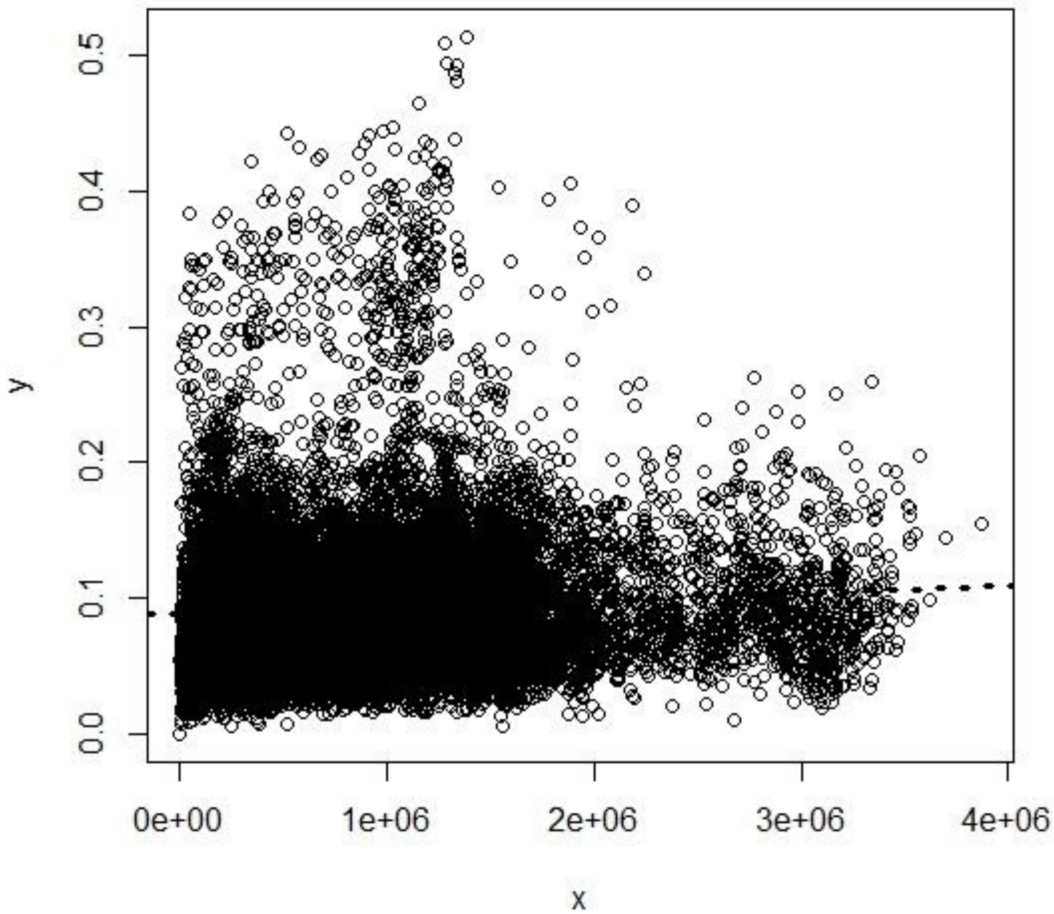

Figure 13: Mantel test: *Rhoicosphenia sp. 1*. Mantel statistic r: 0.05131, Significance: 0.154

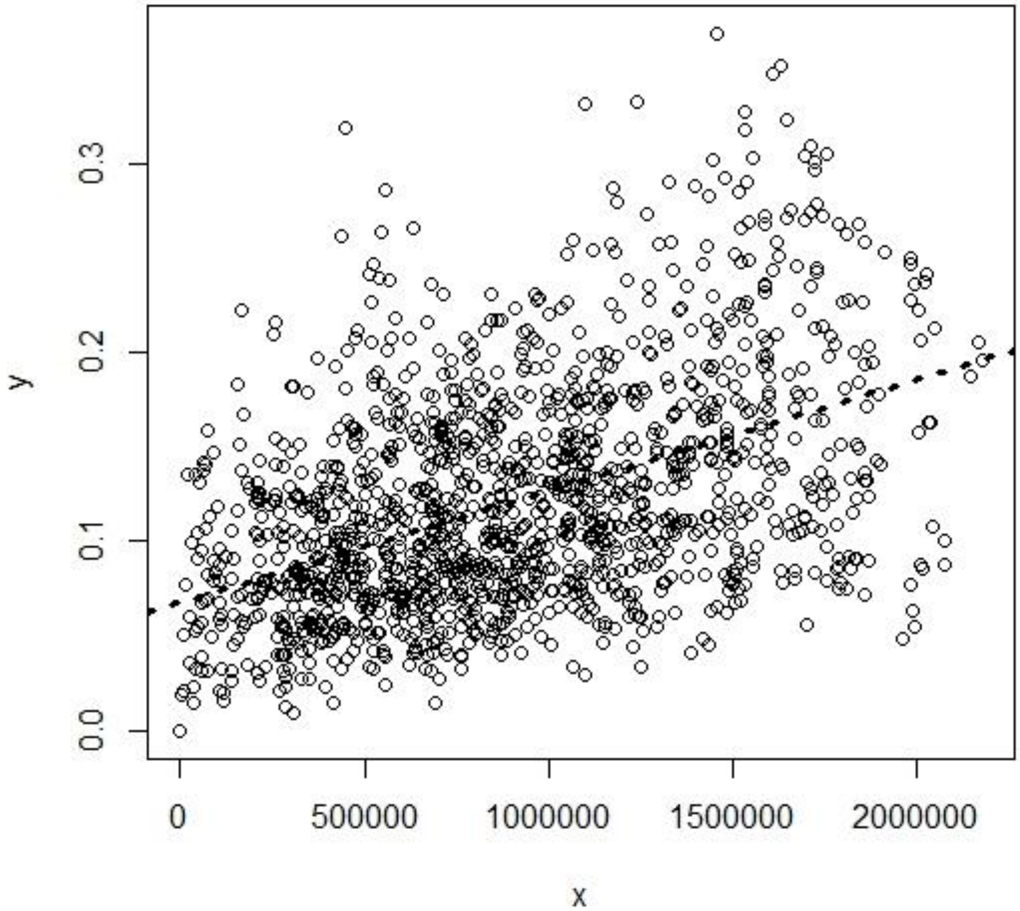

Figure 14: Mantel test: *Rhoicosphenia sp. 2*. Mantel statistic r: 0.4353, Significance: 0.001

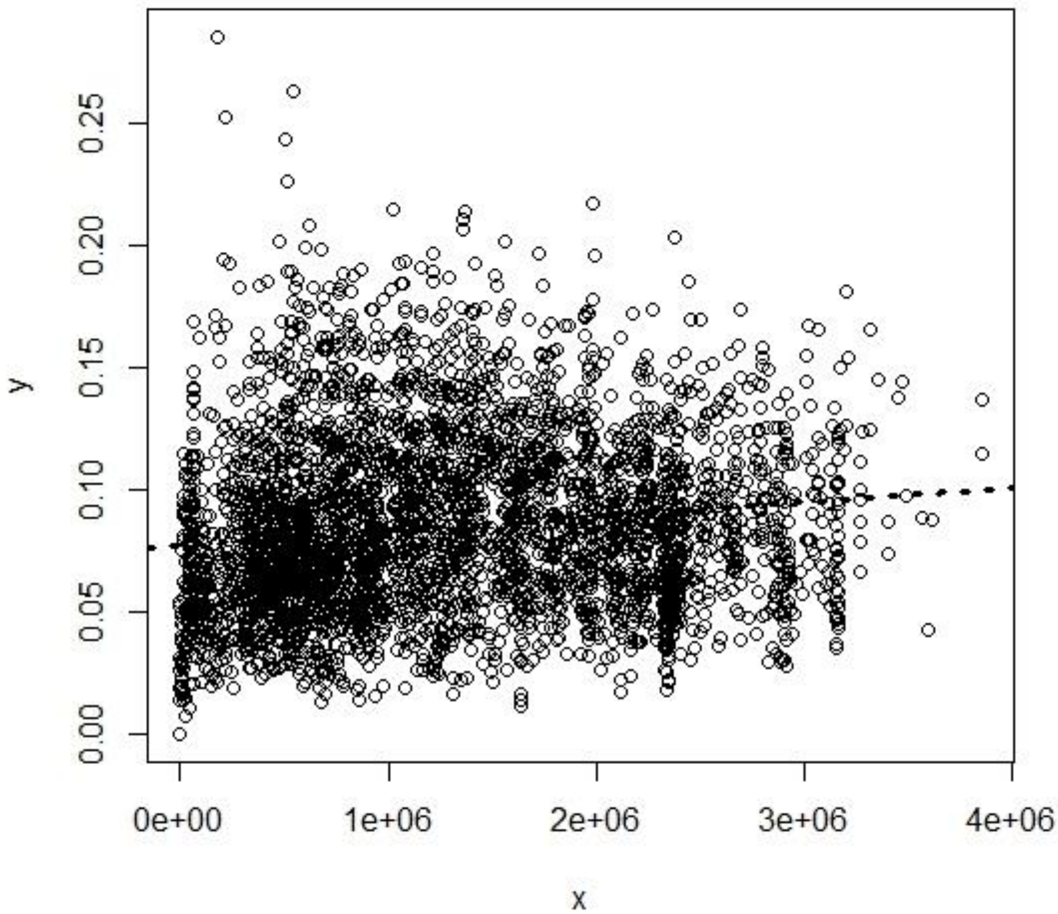

Figure 15: Mantel test: *Rhoicosphenia sp. 3*. Mantel statistic r: 0.09758, Significance: 0.033

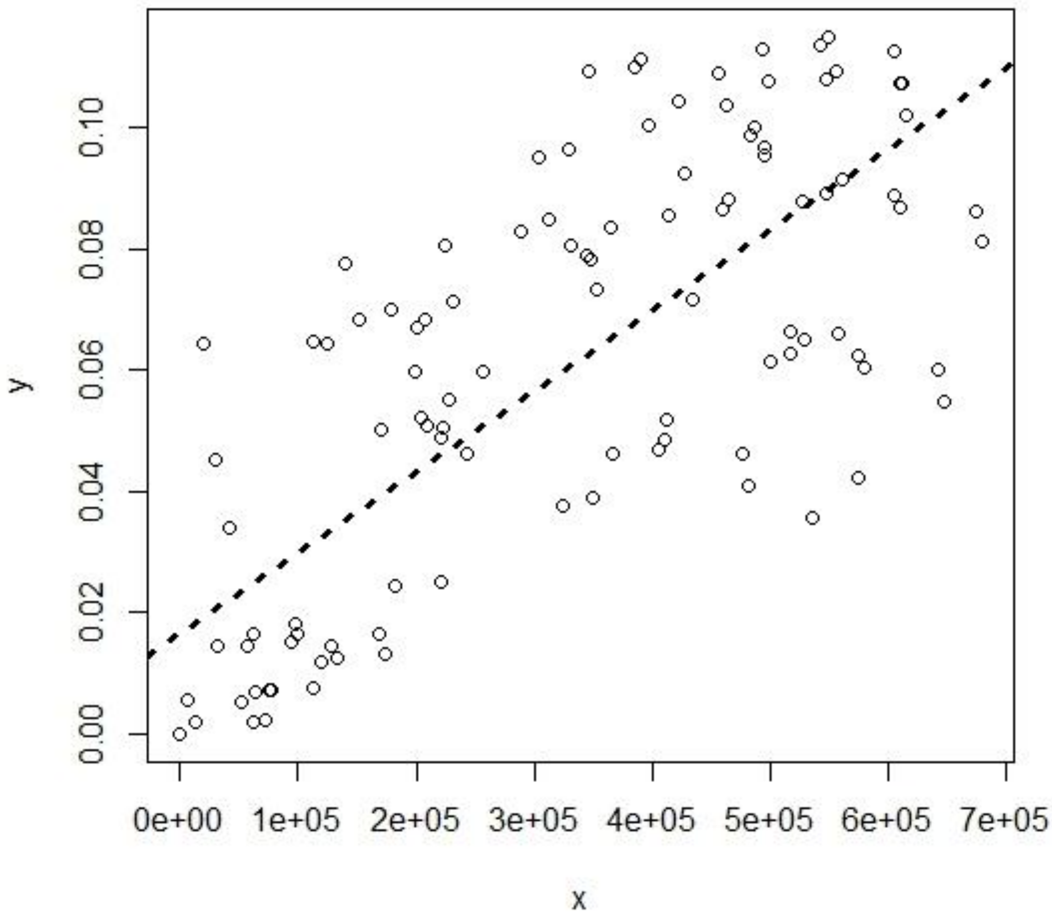

Figure 16: Mantel test: *Rhoicosphenia sp. 4*. Mantel statistic r: 0.7044, Significance: 0.001

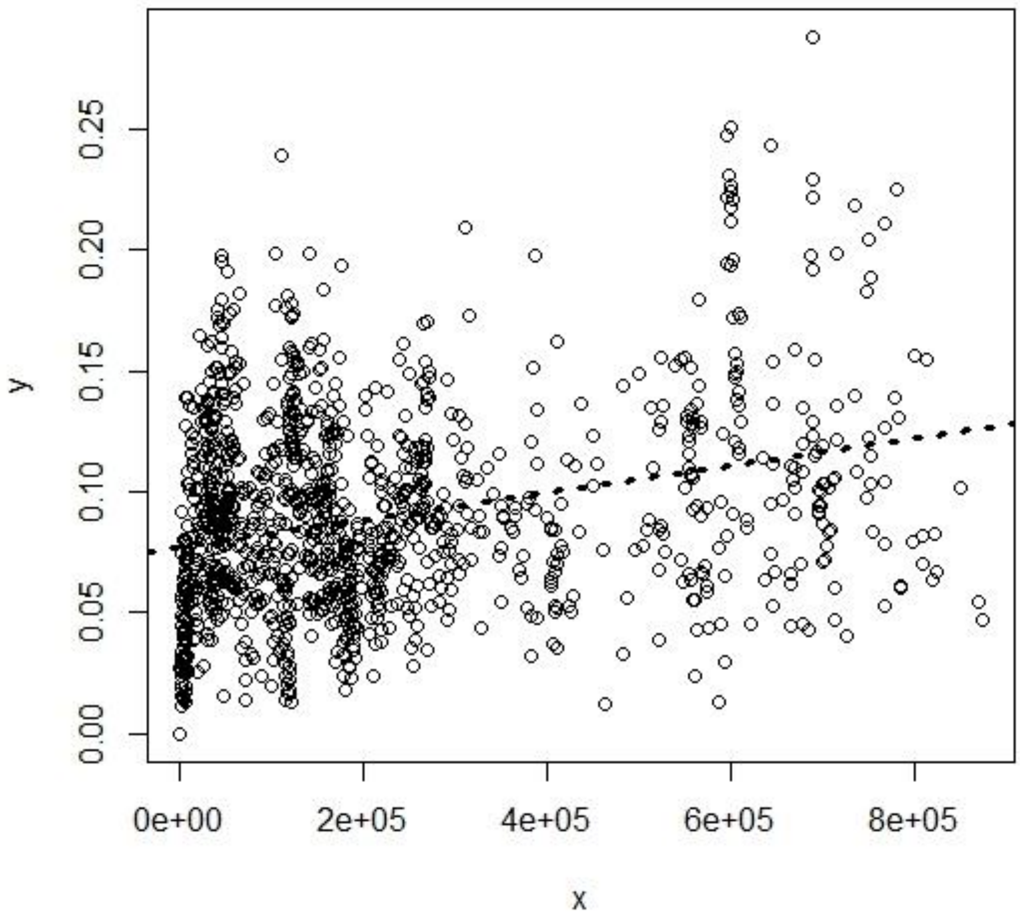

Figure 17: Mantel test: *Rhoicosphenia sp. 5*. Mantel statistic r: 0.2406, Significance: 0.011

Discussion

While the delimitation of diatom species is often restricted to the use of morphological characters and a morphological species concept, other data can be used to support species distinctions (de Queiroz 2007). In the case of the commonly reported diatom genus, *Rhoicosphenia,* ecology and biogeography add more evidence to the delimitation of eight morphologically distinct taxa from across the US as distinct from the 'cosmopolitan' *R. abbreviata* (Kociolek et al. 2015b, Krammer & Lange-Bertalot 1986). Analyses of univariate and multivariate niche space, biogeography, and the combination of ecological and geographical distance support these taxon distinctions.

Biogeography

Results of the mapping of taxon distributions do not support prior notions of one broadly distributed species, as was the case with prior reports of *R. abbreviata* (ANS et al. 2011–2016, Kociolek et al. 2015b). These results are contrary to hypotheses of ubiquitous distributions of microbial eukaryotes (Beijerinck 1913, Baas-Becking 1934, Finlay et al. 2002, Fenchel & Finlay 2004). This study of one genus across a continental scale demonstrates that regionalism and endemism exists in microbial taxon distributions (Martiny et al. 2006). The mapping of *Rhoicosphenia* taxa indicates regionalism in the distribution of these taxa, some with smaller (*R. sp. 4*) and some with larger (*R. sp. 1*, *R. sp. 3*) ranges, but none found across the entire continental US. *Rhoicosphenia sp. 4,* found in Arizona and New Mexico, and *R. californica*, *R. stoermeri* and *R. sp. 5*, found in California, have relatively restricted geographical ranges which is similar to reports of non-*R. abbreviata* species documented in Europe and Asia (Levkov et al. 2010), (Figure 1). Examples of taxa in Europe with restricted ranges are *Rhoicosphenia macedonica* Levkov & Krstic and *Rhoicosphenia tenuis* Levkov & Nakov both known only from

Lake Ohrid, *Rhoicosphenia affinis* found in eutrophic waters of Asia (Levkov et al. 2010), and *Rhoicosphenia baicalensis* Skabichevskii known only from Lake Baikal (Skabichevskii 1976). *Rhoicosphenia lowei* and *R. sp. 2* have slightly broader ranges, being found mainly west of the Great Plains. Finally, *R. sp. 1* and *R. sp. 3* are most widely distributed with ranges spanning the Rocky Mountains in the West, to the Atlantic Coast in the East. The varying degrees of regionalism in the distribution of these taxa, as well as examples of European and Asian species, directly contrast the current view of the most commonly reported species in the genus, *R. abbreviata* (Krammer & Lange-Bertalot 1986), as well as other microbes (Finlay et al. 2002, Fenchel & Finlay 2004). Further, examining the taxa found in California, five taxa are present; *R. californica* is found throughout the state, *R. sp. 5* is more common in southern CA, *R. stoermeri* and *R. lowei* are more common in northern CA, and *R. sp. 2* is found in the eastern central part of the state.

In regards to shared ranges, only 7 of the 28 species pairs are found in the same sites (Table 2). This indicates that, although there is some range overlap, most of the taxa are found to be restricted to certain geographical areas. The regionalism of *Rhoicosphenia* taxa in the US is important for taxonomists working on regional floristics or water quality monitoring. For example, for studies being conducted in the northeastern US, two taxa may be found – either *R. sp. 1* or *R. sp. 3*; in contrast, for studies in CA, there may be up to five *Rhoicosphenia* taxa present. While species diversity has increased, which adds challenges based on the level of taxonomic expertise by the microscopist, the geographic ranges will allow for taxonomists to better understand what they *may* see in a given location, to help to determine what they *are* seeing in that location.

Further, the high diversity of *Rhoicosphenia* is noteworthy – three of the eight taxa from the US, *R. californica*, *R. stoermeri* and *R. sp. 5*, have ranges almost completely restricted to CA, or a good deal of range in CA, as in the case of *R. lowei*. This pattern of diversity is similar to that of vascular plants of the California Floristic Province (CFP), home to over 6,000 taxa of the greater than 16,000 taxa from the US Flora. While the debate between cosmopolitan and endemic diatoms has frequently been addressed in diatom (Bahls 2009, Bahls 2013) and microbial literature (Fenchel & Finlay 2004, Martiny et al. 2006), there has been no mention of diatoms in the context of the CFP. The additional diversity of *Rhoicosphenia* in California and the Western US may provide further impetus for the pursuit of research on whether or not that area (or the CFP) is an area of high endemism for diatoms (Harold & Mooi 1994).

Ecological niche

R. abbreviata and its synonym *R. curvata* are reported globally and are often followed by statements about their broad ecological tolerances (Lowe 1974, Czarnecki & Blinn 1977, 1978, Foged 1984b). The results of these analyses do not support the notion that the different taxa of *Rhoicosphenia* in the US have a broad water chemistry niche. I hypothesize that the reason the niche of *R. abbreviata* (= *R. curvata*) was considered to be broad in US streams is due to the presence of the multiple, newly described and still undescribed taxa being misidentified as *R. abbreviata* in past analyses. These previous studies of *R. abbreviata* were made under the impression that there is high morphological variability within the species, and that size range, striae densities, and other characters used in past identifications were broad (Krammer & Lange-Bertalot 1986). Newly published species and undescribed taxa demonstrate that the diversity of *Rhoicosphenia* is greater, and that the species conform to a more 'modern' morphological species concept used by diatomists (Levkov & Nakov 2008, Levkov et al. 2010, Thomas &

Kociolek 2015, Chapter 2 of this dissertation). An unpublished investigation of the type population of *R. abbreviata* indicates that there is little variability in size and morphology, contrary to the broad variability that previous studies have indicated (Thomas unpublished). The results of this study and the analyses of the ecological data that accompany the species from the US do not support broad ecological or morphological ranges for these new species.

While some taxa included in the analysis (*R. californica*, *R. sp. 1,* and *R. sp. 2*) may look superficially similar, their ecological niches are statistically different. Historically, diatom species have been described based on morphological differences (Round et al. 1990), however slight, but this study shows that ecology may be another useful tool in differentiating taxa. The ANOSIM analysis revealed that many species have distinct niche requirements. Some of the pairs with statistically different niches are morphologically similar to each other (e.g. *R. californica* & *R. sp. 2*; *R. sp. 1* & *R. sp. 2*), while the other pairs of species with statistically different niches are more easily distinguishable based on morphological differences (e.g. *R. sp. 1* & *R. sp. 3*; *R. sp. 1* & *R. lowei*; *R. californica* & *R. sp. 5*; *R. californica* & *R. sp. 3*). One pair of morphologically similar taxa, *R. lowei* and *R. stoermeri*, share a similar niche, however, these taxa are only slightly similar morphologically, mainly due to their large size. One taxon, *R. sp. 4*, shares niche space with all other taxa, with the exception of *R. stoermeri*. This may be because there are relatively few records (n=15) records of this species, and there is not as much statistical support to distinguish its niche from the others. These results are compelling because they provide statistically supported differences in the ecological niches of many taxon pairs. Further, while diatom niche and distribution has been studied (Astorga et al. 2012, Bennett et al. 2010, Vanormelingen et al. 2008), it has been rarely studied in terms of the species within one genus (Potapova & Hamilton 2007).

When the multivariate analysis of niche (NMDS and ANOSIM) was examined for each variable individually (pH, P, Si, Cond, Su), the taxa analyzed did not have the same ranges for each parameter, and when they shared niche space in one parameter, they had divergent requirements for other parameters. In using diatom communities to assess water quality of streams, multivariate techniques are employed – however, in the delimitation of diatom species based on morphology, only qualitative statements of ecology are made, if at all. The results of this study showed that conductivity played a minor role in the delimitation of niche (Table 4), contrary to statements in the literature about conductivity being an important factor in determining diatom species presence (Potapova & Charles 2002). Similarly, in this study pH did not amount to substantial distinctions between taxon pairs (Table 4), which is unusual because pH (similar to conductivity) has often been shown to contribute to the understanding of diatom niche (ter Braak & van Dame 1989). In the cases of conductivity and pH it is possible that due to the log transformation of other variables, their effect in relation to other analyzed variables has been minimized. Many interesting patterns can be found in the univariate descriptive analyses using Box Plots. In terms of pH (Fig. 5), while previous reports of *R. abbreviata* mentioned its tolerance to a broad range of pH conditions, the results of this study show that the eight species found in the US have the majority of their range in rather narrow pH ranges. One example are the high sulfate levels in CA. Recommended sulfate levels in streams should be below 250 mg/L, but the majority of sites with *R. sp. 5* (found in CA) have levels in great excess of that threshold.

The synthesis of the ecological and biogeographic data with Mantel's tests offered insight into the regionalism and range sizes of the *Rhoicosphenia* taxa. All taxa showed a correlation between the two variables, with ecological distance increasing as geographic distance increased (seven of the eight taxa had significant correlations). This relationship suggests that as ecological conditions change over geographic distance, taxa are no longer able to live in the changing environmental conditions. A previous study on continental diatoms and the spatial and environmental gradients of their habitats suggested a regionalism in diatom distributions that was not just based on environmental but also geographic factors (Potapova & Charles 2002). A subsequent study based on diatom distributions, ecology, and geography refined the Environmental Protection Agencies "Nutrient" Ecoregions (based on Level III ecoregions, Omernik 1995) into five diatom ecoregions (Potapova & Charles 2007, Fig. 1). These results suggest that not all diatom taxa are evenly distributed across the US, as is the case with the *Rhoicosphenia* taxa in these analyses, and that the regional nature of the diatom flora in the US could lead to better predictive models of the relationship between diatoms and water chemistry for use in water quality monitoring. While diatoms have been considered cosmopolitan in their distributions in the past (Hustedt 1959, Krammer & Lange-Bertalot 1986), recent investigations (Vyverman et al. 2007, Vanormelingen et al. 2008) have introduced evidence that suggests diatoms may have smaller ranges than previously considered.

General conclusions

The broad niche of *R. abbreviata* (= *R. curvata*) in US streams may be due to the presence of multiple undescribed (new to science) species being misidentified as *R. abbreviata*. Previous studies of *R. abbreviata* were made under the impression that there is high morphological variability within the species, and size and striae densities were broad. This lack of 'good' taxonomic understanding of both rare and common diatoms can inhibit the utility of diatoms in water quality monitoring (Potapova & Charles 2007, Round 2004). Newly published species show that there are many species that adhere to the morphological species concept delimitations that would have formerly been identified as *R. abbreviata*, but are now accepted as different species (Levkov & Nakov 2008, Levkov et al. 2010, Thomas & Kociolek 2015). An investigation into the type population of *R. abbreviata* indicates that there is limited variability in size and morphology, contrary to what previous studies have indicated (Thomas unpublished). The lumping and force-fitting of diatom morphologies into few, broadly circumscribed taxa, as was the case with *Rhoicosphenia* in the US (and globally), prevent growth in our understanding of diatom diversity (Krammer & Lange-Bertalot 1986), distributions (Fenchel & Finlay 2006), and ecological preferences (Vanormelingen et al. 2008).

As the diversity, niche, and phylogeny of *Rhoicosphenia* in the US is further explored it will provide a further refined taxonomy of the genus that can be applied to water quality monitoring, other ecological studies, and general information on diatoms. Further, floristic analyses from various regions of the US and continued water quality monitoring efforts will possibly add more locations and ecological information to the ranges of these taxa. The ultimate goal of this work is to strengthen the application of diatoms in community analyses for water quality monitoring purposes. While *Rhoicosphenia* is only 1 of 97 genera in California, and 3 of approximately 1800 species, it is quite common in the state, being in approximately 80 % of samples. Similarly, *Rhoicosphenia* is also common across the US, and is only one genus and prior to this dissertation one species, in a national list that currently has 158 genera and over 2000 species (ANSP et al. 2011–2016). The implications of this large amount of diversity not examined in this study may mean that while a more complete understanding of *Rhoicosphenia* will not in itself change water quality monitoring studies, it can be used as a model to assess the morphology, ecology, and distribution of other (common) taxa. The success in determining multivariate niche space in this analysis should lead to detailed investigations of the niche requirements of species in other commonly reported genera. A more refined examination of the water chemistry based niche of diatoms and the coupling of that information with detailed taxonomy can only serve to improve our monitoring efforts.

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APPENDIX A

Taxon	Project	Sample ID	Algal Sample ID	Latitude	Longitude	State
R. californica	SWAMP	102PS0139	UCOB_6154	41.99596	-122.95980	California
R. lowei	SWAMP	102PS0139	UCOB_6154	41.99596	-122.95980	California
R. californica	SWAMP	103CDCHHR	UCOB_6302	41.78887	-124.07766	California
R. californica	SWAMP	105PS0067	UCOB_3859	41.13969	-123.13928	California
R. californica	SWAMP	105PS0067	UCOB_3895	41.13969	-123.13928	California
R. californica	SWAMP	105PS0069	UCOB_3915	41.29520	-123.94041	California
R. californica	SWAMP	105PS0188	UCOB_3084	41.71972	-122.34917	California
R. californica	SWAMP	106FS0040	UCOB_7629	40.16904	-123.02523	California
R. californica	SWAMP	106PS0166	UCOB_7331	41.04442	-123.61116	California
R. stoermeri	SWAMP	106WE1079	UCOB_9232	41.13251	-122.80644	California
R. californica	SWAMP	107WER092	UCOB_9233	41.40000	-124.05806	California
R. californica	SWAMP	110ECSLSF	UCOB_7738a	40.67323	-124.09660	California
R. californica	SWAMP	110PS0114	UCOB_6165	40.68788	-124.05053	California
R. lowei	SWAMP	110PS0114	UCOB_6165	40.68788	-124.05053	California
R. californica	SWAMP	110SMCATH	UCOB_7337	40.63848	-124.10397	California
R. lowei	SWAMP	110SMCATH	UCOB_7337	40.63848	-124.10397	California
R. californica	SWAMP	111CE0569	UCOB_6368	40.34610	-123.99327	California
R. californica	SWAMP	111CE0569	UCOB_6369	40.34610	-123.99327	California
R. californica	SWAMP	111PAL105	UCOB_7248	40.34982	-123.96427	California
R. californica	SWAMP	111PS0008	UCOB_3092	39.52196	-123.39670	California
R. stoermeri	SWAMP	111PS0008	UCOB_3092	39.52196	-123.39670	California
R. californica	SWAMP	111PS0057	UCOB_3077	40.46917	-123.92925	California
R. californica	SWAMP	111PS0095	UCOB_3989	39.78908	-123.73694	California
R. stoermeri	SWAMP	111PS0095	UCOB_3903	39.78908	-123.73694	California
R. californica	SWAMP	111PS0110	UCOB_3897	39.35154	-122.87674	California
R. californica	SWAMP	111PS0169	UCOB_7327	40.58297	-123.98891	California
R. californica	SWAMP	111PS0204	UCOB_6168	39.27704	-122.86603	California
R. lowei	SWAMP	111SF1569	UCOB_6042	40.24307	-123.83101	California
R. lowei	SWAMP	111SF1944	UCOB_6041	40.21684	-123.79095	California
R. lowei	SWAMP	111SF2538	UCOB_5956	40.14781	-123.80190	California
R. lowei	SWAMP	111SF2538	UCOB_6040	40.14781	-123.80190	California
R. californica	SWAMP	112PS0157	UCOB_7241	40.23161	-124.11221	California
R. californica	SWAMP	113GAR010	UCOB_3462	38.83737	-123.54644	California
R. californica	SWAMP	113GAR011	UCOB_7683	38.89139	-123.45587	California
R. californica	SWAMP	113GAR110	UCOB_4843	38.85530	-123.56022	California
R. californica	SWAMP	113GAR118	UCOB_5646	38.84264	-123.54917	California
R. californica	SWAMP	113GAR178	UCOB_5708	38.87490	-123.49376	California
R. californica	SWAMP	113GAR244	UCOB_5751	38.93172	-123.59238	California
R. lowei	SWAMP	113GAR244	UCOB_5751	38.93172	-123.59238	California
R. californica	SWAMP	113PS0132	UCOB_6170	39.55671	-123.72350	California
R. stoermeri	SWAMP	114CE0131	UCOB_9230	38.78972	-123.19639	California
R. stoermeri	SWAMP	114WER118	UCOB_2979	38.58750	-123.06222	California
R. californica	SWAMP	201AHO350	UCOB_8736	37.94495	-122.74306	California
R. californica	SWAMP	201LAG335	UCOB_4292	37.99222	-122.66000	California
R. californica	SWAMP	201LAG380	UCOB_3998	37.96722	-122.64945	California
R. californica	SWAMP	202BUT030	UCOB_6313	37.22474	-122.33254	California
R. californica	SWAMP	202BUT040	UCOB_7117	37.24195	-122.31719	California
R. californica	SWAMP	202BUT050	UCOB_4290	37.20607	-122.33483	California

R. californica	SWAMP	202PES162	UCOB_2941	37.26914	-122.26395	California
R. californica	SWAMP	202SPE090	UCOB_3971	37.57500	-122.46300	California
R. lowei	SWAMP	204ALA525	UCOB_8630	37.49664	-121.76043	California
R. californica	SWAMP	204PS0094	UCOB_6173	37.68937	-121.87581	California
R. californica	SWAMP	205AAG400	UCOB_8729	37.37189	-121.73289	California
R. californica	SWAMP	205GCAxxx	UCOB_6315	37.18100	-121.87444	California
R. californica	SWAMP	205LGA700	UCOB_8631	37.11971	-121.90318	California
R. californica	SWAMP	205PS0045	UCOB_3983	37.29359	-121.93299	California
R. californica	SWAMP	205SFC880	UCOB_8735	37.30676	-121.68893	California
R. californica	SWAMP	205STE110	UCOB_3987	37.28710	-122.12600	California
R. californica	SWAMP	205WUN450	UCOB_8731	37.44892	-122.29426	California
R. lowei	SWAMP	205WUN450	UCOB_8731	37.44892	-122.29426	California
R. californica	SWAMP	206BRC020	UCOB_8740	37.92780	-122.15034	California
R. californica	SWAMP	206HCT020	UCOB_8727	38.47124	-122.48879	California
R. californica	SWAMP	206MIL020	UCOB_8716	38.54093	-122.51006	California
R. californica	SWAMP	206NAP090	UCOB_8718	38.41890	-122.35326	California
R. lowei	SWAMP	206NAP090	UCOB_8718	38.41890	-122.35326	California
R. californica	SWAMP	206NAP200	UCOB_8721	38.56873	-122.55527	California
R. californica	SWAMP	206NAP700	UCOB_8737	38.62776	-122.61277	California
R. californica	SWAMP	206RED032	UCOB_8715	38.31785	-122.32750	California
R. lowei	SWAMP	206RED032	UCOB_8715	38.31785	-122.32750	California
R. californica	SWAMP	206SON019	UCOB_8726	38.26225	-122.46270	California
R. lowei	SWAMP	206SON019	UCOB_8726	38.26225	-122.46270	California
R. californica	SWAMP	206SON050	UCOB_8724	38.29840	-122.48120	California
R. lowei	SWAMP	206SON050	UCOB_8724	38.29840	-122.48120	California
R. californica	SWAMP	206SON160	UCOB_8722	38.36376	-122.52617	California
R. lowei	SWAMP	206SON160	UCOB_8722	38.36376	-122.52617	California
R. lowei	SWAMP	206SON210	UCOB_8723	38.40492	-122.55097	California
R. californica	SWAMP	206SON260	UCOB_8720	38.41879	-122.56145	California
R. californica	SWAMP	206SON300	UCOB_8713	38.44264	-122.53139	California
R. californica	SWAMP	206SON320	UCOB_8709	38.43597	-122.50745	California
R. californica	SWAMP	206TUL120	UCOB_7118	38.28377	-122.21725	California
R. lowei	SWAMP	207PS0142	UCOB_7109	37.94989	-121.97286	California
R. californica	SWAMP	304PS0006	UCOB_3027	36.97660	-121.89287	California
R. californica	SWAMP	304PS0006	UCOB_3033	36.97660	-121.89287	California
R. californica	SWAMP	304PS0018	UCOB_3029	37.11937	-122.05035	California
R. californica	SWAMP	304SPC236	UCOB_6318	37.16832	-122.21422	California
R. californica	SWAMP	304WDCAH1	UCOB_6319	37.11372	-122.26978	California
R. californica	SWAMP	305LGCACR	UCOB_2966	36.34867	-120.82087	California
R. californica	SWAMP	305LGCBRC	UCOB_6320	37.14816	-121.77369	California
R. sp. 5	SWAMP	305PS0034	UCOB_3032	36.91609	-121.69873	California
R. californica	SWAMP	305PS0057	UCOB_3957	36.95204	-121.51177	California
R. stoermeri	SWAMP	305PS0061	UCOB_3964	37.08261	-121.60109	California
R. californica	SWAMP	305SSCAUC	UCOB_6321	37.08499	-121.79434	California
R. californica	SWAMP	305UVCASC	UCOB_6322	37.08660	-121.79451	California
R. californica	SWAMP	307CMRADC	UCOB_6323	36.37223	-121.66308	California
R. californica	SWAMP	307SCCARR	UCOB_6324	36.43082	-121.79847	California
R. californica	SWAMP	308BGC	UCOB_7121	36.07091	-121.59807	California
R. californica	SWAMP	308BSU	UCOB_6325	36.24579	-121.77223	California
R. californica	SWAMP	308LSRASC	UCOB_7120	36.32560	-121.78943	California
R. californica	SWAMP	308LSRASC	UCOB_7782	36.32560	-121.78943	California

R. californica	SWAMP	308MWCAH1	UCOB_6326	36.16255	-121.66643	California
R. californica	SWAMP	308PWCAH1	UCOB_6327	35.93555	-121.46781	California
R. californica	SWAMP	308SAM	UCOB_6328	35.81577	-121.35838	California
R. stoermeri	SWAMP	308SAM	UCOB_6328	35.81577	-121.35838	California
R. californica	SWAMP	308SBCAH1	UCOB_6329	36.45510	-121.92261	California
R. californica	SWAMP	309ARSARC	UCOB_6331	36.11966	-121.46866	California
R. californica	SWAMP	309PS0116	UCOB_7104	36.06391	-121.32623	California
R. californica	SWAMP	309PS0116	UCOB_7105	36.06391	-121.32623	California
R. californica	SWAMP	309WLCATC	UCOB_6332	36.21306	-121.53505	California
R. californica	SWAMP	310COO	UCOB_6333	35.25476	-120.88549	California
R. californica	SWAMP	310LPCBPC	UCOB_3920	35.28023	-120.54114	California
R. californica	SWAMP	310OLD	UCOB_6334	35.47167	-120.85895	California
R. californica	SWAMP	310SSU	UCOB_7122	35.60888	-121.07663	California
R. californica	SWAMP	310SSU	UCOB_7780	35.60888	-121.07663	California
R. sp. 5	SWAMP	310SSU	UCOB_7122	35.60888	-121.07663	California
R. sp. 5	SWAMP	310SSU	UCOB_7780	35.60888	-121.07663	California
R. californica	SWAMP	312RYCALR	UCOB_6336	34.67438	-119.29751	California
R. sp. 5	SWAMP	403LNCASC	UCOB_6339	34.53893	-119.16139	California
R. sp. 5	SWAMP	403S00640	UCOB_3782	34.60115	-118.55800	California
R. sp. 5	SWAMP	403S00772	UCOB_3786	34.40976	-118.93220	California
R. sp. 5	SWAMP	403S00831	UCOB_5847	34.43048	-118.83180	California
R. sp. 5	SWAMP	403S01136	UCOB_7203	34.62762	-118.74403	California
R. californica	SWAMP	403S01536	UCOB_7201	34.56909	-118.39213	California
R. sp. 5	SWAMP	403S02764	UCOB_7207	34.44778	-118.75490	California
R. californica	SWAMP	404BA0142	UCOB_2920	34.05144	-118.77622	California
R. californica	SWAMP	404BA0376	UCOB_2922	34.11648	-118.66165	California
R. californica	SWAMP	404BA0526	UCOB_2923	34.04298	-118.87220	California
R. californica	SWAMP	404BA0964	UCOB_2927	34.06133	-118.96491	California
R. californica	SWAMP	404BA1128	UCOB_2928	34.10390	-118.71271	California
R. californica	SWAMP	404BA1144	UCOB_2929	34.06064	-118.63755	California
R. sp. 5	SWAMP	404BA1166	UCOB_3914	34.03762	-118.75038	California
R. sp. 5	SWAMP	404S00808	UCOB_5859	34.11411	-118.77907	California
R. sp. 5	SWAMP	404S02920	UCOB_3760	34.17748	-118.76700	California
R. californica	SWAMP	404S03048	UCOB_3754	34.18426	-118.79089	California
R. sp. 5	SWAMP	404S05992	UCOB_3779	34.15698	-118.75880	California
R. sp. 5	SWAMP	404S06456	UCOB_3785	34.06463	-118.58685	California
R. sp. 5	SWAMP	404S08616	UCOB_3778	34.12188	-118.79240	California
R. californica	SWAMP	404S13416	UCOB_7200	34.09875	-118.71595	California
R. californica	SWAMP	404S14952	UCOB_7206	34.14268	-118.70090	California
R. sp. 5	SWAMP	404S14952	UCOB_7206	34.14268	-118.70090	California
R. sp. 5	SWAMP	404S16232	UCOB_7197	34.12550	-118.75317	California
R. sp. 5	SWAMP	404S16516	UCOB_3788	34.12998	-118.75648	California
R. californica	SWAMP	404S17664	UCOB_3757	34.14994	-118.69760	California
R. sp. 5	SWAMP	404S17664	UCOB_3757	34.14994	-118.69760	California
R. sp. 5	SWAMP	404S18666	UCOB_7205	34.17207	-118.76376	California
R. sp. 5	SWAMP	404S28270	UCOB_8782	34.13663	-118.75726	California
R. sp. 5	SWAMP	404S31468	UCOB_8780	34.16874	-118.76171	California
R. californica	SWAMP	404S34120	UCOB_8787	34.16559	-118.78919	California
R. sp. 5	SWAMP	404S34120	UCOB_8787	34.16559	-118.78919	California
R. californica	SWAMP	408BA0836	UCOB_2935	34.19072	-119.00511	California
R. sp. 5	SWAMP	412PS0040	UCOB_3014	34.26448	-118.48787	California

R. californica	SWAMP	504PS0019	UCOB_3046	39.72855	-121.88105	California
R. lowei	SWAMP	504PS0019	UCOB_3046	39.72855	-121.88105	California
R. californica	SWAMP	506PS0003	UCOB_3049	40.94366	-122.33472	California
R. californica	SWAMP	506PS0003	UCOB_3940	40.94366	-122.33472	California
R. californica	SWAMP	506PS0003	UCOB_6182	40.94366	-122.33472	California
R. californica	SWAMP	507PS0122	UCOB_3974	40.39458	-121.93617	California
R. lowei	SWAMP	507PS0122	UCOB_3974	40.39458	-121.93617	California
R. californica	SWAMP	507PS0286	UCOB_7237	40.50043	-121.93300	California
R. californica	SWAMP	507PS0314	UCOB_7727	40.42523	-121.99229	California
R. lowei	SWAMP	507PS0314	UCOB_7727	40.42523	-121.99229	California
R. californica	SWAMP	509ACNFPP	UCOB_3004	40.24167	-121.86250	California
R. californica	SWAMP	509BCCBPW	UCOB_3916	39.86971	-121.70748	California
R. lowei	SWAMP	509BCCBPW	UCOB_3916	39.86971	-121.70748	California
R. californica	SWAMP	509BSCADC	UCOB_7632	40.10009	-121.66986	California
R. lowei	SWAMP	509CWCPLR	UCOB_2957	40.20959	-121.92700	California
R. californica	SWAMP	509DCPWxx	UCOB_2981	40.06961	-121.70886	California
R. lowei	SWAMP	509DCPWxx	UCOB_2981	40.06961	-121.70886	California
R. californica	SWAMP	509DRCBPC	UCOB_7631	40.16590	-121.60068	California
R. californica	SWAMP	509ICPPCX	UCOB_2971	40.23333	-121.87889	California
R. californica	SWAMP	509ICPPCX	UCOB_2991	40.23333	-121.87889	California
R. lowei	SWAMP	509ICPPCX	UCOB_2971	40.23333	-121.87889	California
R. lowei	SWAMP	509ICPPCX	UCOB_2991	40.23333	-121.87889	California
R. californica	SWAMP	509PCDTWR	UCOB_2986	40.31194	-121.88583	California
R. lowei	SWAMP	509PCDTWR	UCOB_2965	40.31194	-121.88583	California
R. lowei	SWAMP	509PCDTWR	UCOB_2986	40.31194	-121.88583	California
R. californica	SWAMP	509PS0085	UCOB_7732	40.25377	-121.56050	California
R. californica	SWAMP	509PS0170	UCOB_6186	40.26488	-121.76810	California
R. californica	SWAMP	513PS0008	UCOB_3041	38.93275	-122.93287	California
R. californica	SWAMP	513PS0008	UCOB_3069	38.93275	-122.93287	California
R. californica	SWAMP	513PS0008	UCOB_6189	38.93275	-122.93287	California
R. stoermeri	SWAMP	513PS0008	UCOB_3041	38.93275	-122.93287	California
R. stoermeri	SWAMP	513PS0008	UCOB_3069	38.93275	-122.93287	California
R. stoermeri	SWAMP	513PS0008	UCOB_6189	38.93275	-122.93287	California
R. lowei	SWAMP	513UNCAKC	UCOB_2990	39.16643	-122.64117	California
R. californica	SWAMP	516PS0287	UCOB_7729	39.04512	-121.11640	California
R. californica	SWAMP	517PS0039	UCOB_4287	39.50109	-121.28704	California
R. californica	SWAMP	518PS0045	UCOB_3866	40.00373	-120.93305	California
R. californica	SWAMP	518SED086	UCOB_7630	39.80619	-121.04260	California
R. lowei	SWAMP	518SED086	UCOB_7630	39.80619	-121.04260	California
R. californica	SWAMP	518YLCAFR	UCOB_7624	40.01207	-121.24751	California
R. lowei	SWAMP	518YLCAFR	UCOB_7624	40.01207	-121.24751	California
R. californica	SWAMP	519MNRASR	UCOB_2959	38.75896	-121.25594	California
R. californica	SWAMP	519PS0402	UCOB_6200	38.79870	-121.34790	California
R. stoermeri	SWAMP	519PS0402	UCOB_6200	38.79870	-121.34790	California
R. lowei	SWAMP	520PS0071	UCOB_3065	39.70008	-121.84953	California
R. californica	SWAMP	520PS0135	UCOB_3954	39.64700	-121.64203	California
R. californica	SWAMP	521LCCBSR	UCOB_6345	39.88531	-121.65056	California
R. californica	SWAMP	526PS0072	UCOB_3098	41.13422	-120.80025	California
R. californica	SWAMP	526PS0072	UCOB_4294	41.13422	-120.80025	California
R. californica	SWAMP	526PS0072	UCOB_6204	41.13422	-120.80025	California
R. lowei	SWAMP	526PS0072	UCOB_3098	41.13422	-120.80025	California

R. lowei	SWAMP	526PS0072	UCOB_4294	41.13422	-120.80025	California
R. lowei	SWAMP	526PS0072	UCOB_6204	41.13422	-120.80025	California
R. californica	SWAMP	526PS0220	UCOB_3081	41.00853	-121.65023	California
R. lowei	SWAMP	526PS0220	UCOB_3081	41.00853	-121.65023	California
R. californica	SWAMP	526PS0948	UCOB_7242	41.20806	-120.93283	California
R. lowei	SWAMP	526PS0948	UCOB_7242	41.20806	-120.93283	California
R. californica	SWAMP	532PS0062	UCOB_6212	38.67892	-120.66779	California
R. californica	SWAMP	532PS0071	UCOB_7734	38.49028	-120.39647	California
R. californica	SWAMP	532PS0071	UCOB_7788	38.49028	-120.39647	California
R. californica	SWAMP	532TGRUPX	UCOB_2997	38.48525	-120.44688	California
R. californica	SWAMP	534ANCACF	UCOB_6346	38.40735	-119.79936	California
R. californica	SWAMP	534RSCAGG	UCOB_7722	38.13194	-120.22041	California
R. lowei	SWAMP	542DPCDPC	UCOB_3001	37.42083	-121.37347	California
R. californica	SWAMP	554PS0160	UCOB_3067	35.46624	-118.32580	California
R. californica	SWAMP	555PS0064	UCOB_3031	36.18281	-118.78852	California
R. californica	SWAMP	603DDM005	UCOB_7718	37.70169	-119.03358	California
R. californica	SWAMP	603PS0044	UCOB_8469	37.12530	-118.35254	California
R. californica	SWAMP	620PS0669	UCOB_6222	36.31991	-117.52579	California
R. sp. 5	SWAMP	620PS0669	UCOB_6222	36.31991	-117.52579	California
R. californica	SWAMP	626PS0619	UCOB_6223	34.41876	-117.97471	California
R. californica	SWAMP	631PS0023	UCOB_3956	38.47279	-119.35211	California
R. californica	SWAMP	632HEN001	UCOB_6354	38.66056	-119.62764	California
R. californica	SWAMP	632MUR001	UCOB_7717	38.49190	-119.70170	California
R. californica	SWAMP	632NOB001	UCOB_6355	38.57340	-119.78967	California
R. californica	SWAMP	632PS0007	UCOB_3057	38.53281	-119.59393	California
R. californica	SWAMP	634PS0062	UCOB_7728	38.87503	-119.97165	California
R. californica	SWAMP	634WSN001	UCOB_7741	39.22273	-120.10026	California
R. californica	SWAMP	637PS0018	UCOB_3066	40.36057	-120.80557	California
R. sp. 5	SWAMP	637PS0018	UCOB_3066	40.36057	-120.80557	California
R. californica	SWAMP	719WWRAEF	UCOB_6356	34.06322	-116.82029	California
R. californica	SWAMP	719WWRAEF	UCOB_6357	34.06322	-116.82029	California
R. californica	SWAMP	722PS0535	UCOB_3943	33.36922	-116.42223	California
R. californica	SWAMP	801CCWFAC	UCOB_7126	34.19020	-117.18227	California
R. californica	SWAMP	801WE1132	UCOB_3928	34.13332	-116.84289	California
R. californica	SWAMP	802FMCAIP	UCOB_7128	33.80722	-116.74250	California
R. californica	SWAMP	845PS0011	UCOB_3010	33.88139	-117.89643	California
R. sp. 5	SWAMP	845PS0011	UCOB_3010	33.88139	-117.89643	California
R. californica	SWAMP	901PS0057	UCOB_3009	33.52866	-117.66925	California
R. sp. 5	SWAMP	901S02702	UCOB_5796	33.51637	-117.74062	California
R. californica	SWAMP	901S04409	UCOB_7131	33.60348	-117.45315	California
R. californica	SWAMP	901S04565	UCOB_7132	33.53161	-117.41415	California
R. sp. 5	SWAMP	901S06798	UCOB_7133	33.53154	-117.74145	California
R. californica	SWAMP	901S06969	UCOB_8748	33.55335	-117.39580	California
R. sp. 5	SWAMP	902S02293	UCOB_5801	33.42340	-117.20467	California
R. sp. 5	SWAMP	902S02357	UCOB_5802	33.40569	-117.24977	California
R. sp. 5	SWAMP	902S05173	UCOB_7135	33.39711	-117.28365	California
R. sp. 5	SWAMP	903SLKYS3	UCOB_2888	33.28908	-117.07136	California
R. californica	SWAMP	904PS0034	UCOB_3008	33.06756	-117.26276	California
R. sp. 5	SWAMP	904PS0034	UCOB_3008	33.06756	-117.26276	California
R. sp. 5	SWAMP	904S00537	UCOB_3791	33.05032	-117.22429	California
R. californica	SWAMP	904S02201	UCOB_5803	33.17980	-117.33735	California

R. sp. 5	SWAMP	904S02201	UCOB_5803	33.17980	-117.33735	California
R. sp. 5	SWAMP	904S02585	UCOB_5805	33.09087	-117.24470	California
R. californica	SWAMP	905S02561	UCOB_7136	33.12974	-116.63592	California
R. californica	SWAMP	907CCCR02	UCOB_6359	33.00222	-116.70889	California
R. californica	SWAMP	907CCCR02	UCOB_6363	33.00222	-116.70889	California
R. californica	SWAMP	907CCCR02	UCOB_6364	33.00222	-116.70889	California
R. californica	SWAMP	907S03210	UCOB_7138	33.00313	-116.72919	California
R. californica	SWAMP	907S05514	UCOB_7139	32.97974	-116.74247	California
R. californica	SWAMP	909JPCH79	UCOB_6358	32.91849	-116.57176	California
R. californica	SWAMP	909JQCASR	UCOB_7116	32.91468	-116.56562	California
R. sp. 5	SWAMP	909SSWR08	UCOB_2883	32.65897	-117.04181	California
R. sp. 5	SWAMP	910S14762	UCOB_5813	32.64788	-116.86999	California
R. californica	SWAMP	911KCKCRx	UCOB_7113	32.78747	-116.45161	California
R. californica	SWAMP	911S01142	UCOB_7140	32.73548	-116.65268	California
R. californica	SWAMP	911S03354	UCOB_3809	32.79112	-116.61677	California
R. sp. 5	SWAMP	911S04086	UCOB_3810	32.67455	-116.57778	California
R. californica	SWAMP	911TCCTCx	UCOB_7114	32.80778	-116.44000	California
R. californica	SWAMP	911TJWIL3	UCOB_7125	32.69361	-116.69528	California
R. sp. 5	SCB	AHAH1	UCOB_2465	34.48340	-120.14157	California
R. sp. 5	SCB	AHAH1	UCOB_2665	34.48340	-120.14157	California
R. californica	SCB	DGKC1	UCOB_2671	33.06730	-117.06599	California
R. californica	SCB	DGSY1	UCOB_2650	33.12794	-116.67616	California
R. sp. 3	US	FW08AZ008	NRSA0562	32.87101	-109.19813	Arizona
R. sp. 4	US	FW08AZ034	NRSA1202	36.08919	-113.25407	Arizona
R. sp. 4	US	FW08AZ045	NRSA1205	36.30101	-112.49462	Arizona
R. sp. 2	US	FW08AZ073	NRSA1210	36.05593	-111.99766	Arizona
R. sp. 4	US	FW08AZ073	NRSA1210	36.05593	-111.99766	Arizona
R. sp. 4	US	FW08AZ077	NRSA1209	36.31936	-111.86289	Arizona
R. sp. 4	US	FW08AZ087	NRSA0676	33.61772	-110.91103	Arizona
R. sp. 4	US	FW08AZ093	NRSA1214	36.84695	-111.61694	Arizona
R. sp. 4	US	FW08AZ098	NRSA1204	36.09844	-113.31690	Arizona
R. sp. 4	US	FW08AZ109	NRSA1203	36.40031	-112.55616	Arizona
R. sp. 3	US	FW08AZ134	NRSA0565	32.89332	-109.79408	Arizona
R. californica	US	FW08CA008	NRSA1181	40.62711	-123.37408	California
R. lowei	US	FW08CA008	NRSA1181	40.62711	-123.37408	California
R. californica	US	FW08CA016	NRSA0538	37.32878	-121.67490	California
R. stoermeri	US	FW08CA016	NRSA0538	37.32878	-121.67490	California
R. californica	US	FW08CA022	NRSA1183	34.35671	-119.01988	California
R. stoermeri	US	FW08CA022	NRSA1183	34.35671	-119.01988	California
R. californica	US	FW08CA075	NRSA0540	39.90521	-121.04656	California
R. californica	US	FW08CA097	NRSA1188	38.27113	-119.33165	California
R. californica	US	FW08CA132	NRSA1191	41.95444	-122.66163	California
R. californica	US	FW08CA168	NRSA1194	41.64846	-124.08845	California
R. stoermeri	US	FW08CA199	NRSA1197	38.83028	-122.90607	California
R. californica	US	FW08CA207	NRSA1198	40.10651	-123.79382	California
R. sp. 3	US	FW08CO001	NRSA0049	37.36588	-108.59328	Colorado
R. sp. 3	US	FW08CO014	NRSA0059	39.89247	-105.05654	Colorado
R. sp. 3	US	FW08CO025	NRSA0047	38.86531	-108.39814	Colorado
R. sp. 1	US	FW08CO031	NRSA0046	40.15970	-105.11843	Colorado
R. sp. 2	US	FW08CO062	NRSA0922	39.95764	-106.54976	Colorado
R. sp. 3	US	FW08CO125	NRSA0632	37.58700	-104.83863	Colorado

R. sp. 1	US	FW08CT004	NRSA1084	41.39887	-73.38665	Connecticut
R. sp. 1	US	FW08CT005	NRSA1088	41.89123	-72.66210	Connecticut
R. sp. 1	US	FW08CT009	NRSA0476	41.63415	-72.74884	Connecticut
R. sp. 1	US	FW08CT015	NRSA0878	41.64140	-73.47779	Connecticut
R. sp. 1	US	FW08CT016	NRSA1091	41.84448	-72.63200	Connecticut
R. sp. 1	US	FW08DE009	NRSA0574	39.83430	-75.57709	Delaware
R. sp. 1	US	FW08DE013	NRSA0415	39.73029	-75.59800	Delaware
R. sp. 1	US	FW08DE025	NRSA1066	39.80614	-75.46541	Delaware
R. lowei	US	FW08ID017	NRSA0849	45.36948	-114.28991	Idaho
R. sp. 2	US	FW08ID017	NRSA0849	45.36948	-114.28991	Idaho
R. sp. 2	US	FW08ID024	NRSA0027	42.17904	-114.22196	Idaho
R. lowei	US	FW08ID044	NRSA0026	42.75271	-116.07437	Idaho
R. sp. 2	US	FW08ID044	NRSA0026	42.75271	-116.07437	Idaho
R. lowei	US	FW08ID049	NRSA0909	45.39650	-114.16045	Idaho
R. sp. 2	US	FW08ID049	NRSA0909	45.39650	-114.16045	Idaho
R. sp. 1	US	FW08MD004	NRSA0065	39.64848	-77.18089	Maryland
R. sp. 1	US	FW08MD008	NRSA0068	39.06637	-77.38957	Maryland
R. sp. 3	US	FW08MD016	NRSA0946	39.64986	-77.84048	Maryland
R. sp. 1	US	FW08ME013	NRSA0453	47.13183	-67.89810	Maine
R. sp. 1	US	FW08MT002	NRSA0312	45.14161	-109.03994	Montana
R. sp. 3	US	FW08MT002	NRSA0312	45.14161	-109.03994	Montana
R. sp. 3	US	FW08MT035	NRSA0091	44.97626	-112.99659	Montana
R. sp. 3	US	FW08MT036	NRSA1235	48.56932	-112.90009	Montana
R. sp. 3	US	FW08MT049	NRSA1234	48.00521	-105.90923	Montana
R. sp. 3	US	FW08MT053	NRSA1113	48.72955	-105.44116	Montana
R. sp. 2	US	FW08MT080	NRSA1226	45.05171	-105.21429	Montana
R. sp. 3	US	FW08MT088	NRSA0796	48.54947	-109.39168	Montana
R. sp. 2	US	FW08ND003	NRSA0175	48.06338	-100.92094	North Dakota
R. sp. 3	US	FW08ND003	NRSA0175	48.06338	-100.92094	North Dakota
R. sp. 2	US	FW08ND004	NRSA0164	47.45974	-96.87662	North Dakota
R. sp. 2	US	FW08ND013	NRSA0184	46.47800	-102.24053	North Dakota
R. sp. 2	US	FW08ND017	NRSA0167	47.25585	-101.80840	North Dakota
R. sp. 3	US	FW08ND017	NRSA0167	47.25585	-101.80840	North Dakota
R. sp. 2	US	FW08ND027	NRSA0179	47.50223	-97.33886	North Dakota
R. sp. 3	US	FW08ND027	NRSA0179	47.50223	-97.33886	North Dakota
R. sp. 2	US	FW08ND028	NRSA0158	46.79972	-101.10684	North Dakota
R. sp. 3	US	FW08ND028	NRSA0158	46.79972	-101.10684	North Dakota
R. sp. 2	US	FW08ND033	NRSA0147	47.04646	-101.10470	North Dakota
R. sp. 3	US	FW08ND033	NRSA0147	47.04646	-101.10470	North Dakota
R. sp. 2	US	FW08ND035	NRSA0178	48.43887	-97.43963	North Dakota
R. sp. 3	US	FW08ND035	NRSA0178	48.43887	-97.43963	North Dakota
R. sp. 2	US	FW08ND066	NRSA0965	48.36719	-102.77780	North Dakota
R. sp. 3	US	FW08ND066	NRSA0965	48.36719	-102.77780	North Dakota
R. sp. 2	US	FW08ND161	NRSA0964	47.31478	-100.91521	North Dakota
R. sp. 1	US	FW08NJ002	NRSA0020	40.81494	-75.04027	New Jersey
R. sp. 1	US	FW08NJ005	NRSA0727	40.50890	-74.46615	New Jersey
R. sp. 1	US	FW08NJ007	NRSA0729	40.91251	-74.18684	New Jersey
R. sp. 1	US	FW08NJ021	NRSA0024	40.62462	-74.47444	New Jersey
R. sp. 4	US	FW08NM022	NRSA0687	36.70793	-108.21145	New Mexico
R. sp. 4	US	FW08NM024	NRSA0690	35.16802	-106.65810	New Mexico
R. californica	US	FW08NM035	NRSA0548	33.20253	-108.20881	New Mexico

R. sp. 4	US	FW08NM038	NRSA0686	36.69929	-107.98527	New Mexico
R. sp. 2	US	FW08NV003	NRSA0211	40.31658	-116.90153	Nevada
R. sp. 2	US	FW08NV004	NRSA0223	40.90311	-115.22426	Nevada
R. sp. 2	US	FW08NV009	NRSA0220	38.32751	-114.27746	Nevada
R. lowei	US	FW08NV011	NRSA0225	41.21399	-116.39872	Nevada
R. sp. 2	US	FW08NV011	NRSA0225	41.21399	-116.39872	Nevada
R. sp. 2	US	FW08NV028	NRSA0244	41.40884	-118.91090	Nevada
R. californica	US	FW08NV040	NRSA0207	39.08216	-119.75730	Nevada
R. sp. 5	US	FW08NV040	NRSA0207	39.08216	-119.75730	Nevada
R. sp. 2	US	FW08NV049	NRSA0252	38.85055	-116.60486	Nevada
R. sp. 5	US	FW08NV050	NRSA0215	40.41024	-118.31544	Nevada
R. californica	US	FW08NV053	NRSA0230	41.95777	-115.86383	Nevada
R. californica	US	FW08NV065	NRSA0251	38.78148	-117.34017	Nevada
R. sp. 1	US	FW08NY015	NRSA0022	42.50112	-74.43891	New York
R. sp. 3	US	FW08NY022	NRSA0019	43.00711	-76.68105	New York
R. sp. 1	US	FW08NY034	NRSA0393	43.13767	-76.29551	New York
R. sp. 1	US	FW08NY050	NRSA0427	43.30450	-76.39770	New York
R. sp. 3	US	FW08NY050	NRSA0427	43.30450	-76.39770	New York
R. sp. 1	US	FW08NY077	NRSA0426	42.74127	-76.47440	New York
R. lowei	US	FW08OR005	NRSA0368	44.89843	-117.42416	Oregon
R. lowei	US	FW08OR006	NRSA0349	43.87510	-123.50258	Oregon
R. sp. 2	US	FW08OR009	NRSA0373	45.30045	-123.47747	Oregon
R. lowei	US	FW08OR015	NRSA0359	42.41324	-123.15797	Oregon
R. californica	US	FW08OR028	NRSA0845	45.48478	-122.95994	Oregon
R. lowei	US	FW08OR043	NRSA0354	43.61499	-122.76646	Oregon
R. lowei	US	FW08OR054	NRSA0357	42.46828	-124.34534	Oregon
R. lowei	US	FW08OR055	NRSA0628	45.73065	-122.92959	Oregon
R. lowei	US	FW08OR058	NRSA0367	44.14641	-122.57705	Washington
R. sp. 2	US	FW08OR058	NRSA0367	44.14641	-122.57705	Washington
R. lowei	US	FW08OR059	NRSA0365	43.57753	-123.50166	Oregon
R. lowei	US	FW08OR064	NRSA1039	45.28694	-116.67222	Oregon
R. sp. 1	US	FW08PA006	NRSA0779	40.37668	-75.53031	Pennsylvania
R. sp. 1	US	FW08PA019	NRSA0903	40.49180	-76.94813	Pennsylvania
R. sp. 1	US	FW08PA021	NRSA0904	41.96990	-76.51192	Pennsylvania
R. sp. 1	US	FW08PA024	NRSA1052	41.02419	-80.16323	Pennsylvania
R. sp. 1	US	FW08PA027	NRSA1042	39.77420	-77.15679	Pennsylvania
R. sp. 1	US	FW08PA029	NRSA0186	41.44305	-75.66055	Pennsylvania
R. sp. 1	US	FW08PA030	NRSA1237	40.14318	-75.51026	Pennsylvania
R. sp. 1	US	FW08PA038	NRSA0841	40.55195	-78.09302	Pennsylvania
R. sp. 1	US	FW08PA044	NRSA1050	40.65367	-79.94249	Pennsylvania
R. sp. 1	US	FW08PA053	NRSA1058	41.95442	-75.84874	Pennsylvania
R. sp. 1	US	FW08PA061	NRSA1059	41.43309	-74.97772	Pennsylvania
R. sp. 4	US	FW08RAZ9020	NRSA0670	48.82927	-100.06735	Arizona
R. sp. 1	US	FW08RI006	NRSA0862	41.99036	-71.49176	Rhode Island
R. sp. 2	US	FW08RND9138	NRSA0271	48.82927	-100.06735	North Dakota
R. sp. 3	US	FW08SD001	NRSA0825	45.40523	-98.05886	South Dakota
R. sp. 3	US	FW08SD011	NRSA0987	44.72564	-103.97010	South Dakota
R. sp. 2	US	FW08SD019	NRSA0114	43.38209	-102.38960	South Dakota
R. sp. 2	US	FW08UT003	NRSA0302	37.55194	-113.40907	Utah
R. sp. 3	US	FW08UT003	NRSA0302	37.55194	-113.40907	Utah
R. sp. 3	US	FW08UT007	NRSA0293	38.33326	-112.18543	Utah

R. sp. 1	US	FW08UT009	NRSA0300	37.31865	-113.71687	Utah
R. sp. 3	US	FW08UT014	NRSA1162	37.22372	-109.20869	Utah
R. sp. 3	US	FW08UT023	NRSA1173	38.35000	-109.75000	Utah
R. sp. 1	US	FW08UT030	NRSA1159	37.27503	-109.43707	Utah
R. sp. 3	US	FW08UT030	NRSA1159	37.27503	-109.43707	Utah
R. sp. 3	US	FW08UT046	NRSA1168	37.19440	-109.73348	Utah
R. lowei	US	FW08UT053	NRSA0306	38.18736	-112.09467	Utah
R. sp. 1	US	FW08UT053	NRSA0306	38.18736	-112.09467	Utah
R. sp. 3	US	FW08UT053	NRSA0306	38.18736	-112.09467	Utah
R. sp. 1	US	FW08VA001	NRSA0509	37.88508	-79.15866	Virginia
R. sp. 1	US	FW08VA041	NRSA1206	36.61999	-82.82102	Virginia
R. lowei	US	FW08WA016	NRSA1026	45.69861	-120.41753	Washington
R. lowei	US	FW08WA036	NRSA1025	46.65599	-117.41411	Washington
R. sp. 2	US	FW08WA036	NRSA1025	46.65599	-117.41411	Washington
R. lowei	US	FW08WA078	NRSA1149	46.53201	-123.82702	Washington
R. lowei	US	FW08WA122	NRSA1148	47.13550	-123.77687	Washington
R. sp. 1	US	FW08WV002	NRSA1006	38.75219	-80.94072	West Virginia
R. sp. 1	US	FW08WV025	NRSA0659	38.46936	-82.27878	West Virginia
R. sp. 2	US	FW08WY005	NRSA0576	44.30322	-106.93893	Wyoming
R. sp. 3	US	FW08WY005	NRSA0576	44.30322	-106.93893	Wyoming
R. sp. 3	US	FW08WY016	NRSA0757	42.85202	-106.18585	Wyoming
R. sp. 3	US	FW08WY025	NRSA0655	44.81826	-107.04476	Wyoming
R. sp. 2	US	FW08WY039	NRSA0751	42.82884	-106.36679	Wyoming
R. sp. 3	US	FW08WY039	NRSA0751	42.82884	-106.36679	Wyoming
R. sp. 2	US	FW08WY041	NRSA0575	44.15839	-106.91202	Wyoming
R. sp. 3	US	FW08WY042	NRSA0334	44.38685	-104.67731	Wyoming
R. sp. 3	US	FW08WY057	NRSA0591	43.94113	-108.04898	Wyoming
R. sp. 3	US	FW08WY071	NRSA0755	42.68898	-105.38901	Wyoming
R. sp. 1	US	GS01102345	GSN00871	42.46940	-71.00750	Massachusetts
R. sp. 1	US	GS01127400	GS017363	41.57080	-72.13360	Connecticut
R. sp. 1	US	GS01184100	GS017254	41.96100	-72.71100	Connecticut
R. sp. 1	US	GS01184490	GS017443	41.91400	-72.55000	Connecticut
R. sp. 1	US	GS01192883	GS017273	41.52000	-72.70600	Connecticut
R. sp. 1	US	GS01196580	GS017283	41.36900	-72.84200	Connecticut
R. sp. 1	US	GS01200000	GS017163	41.65890	-73.52890	New York
R. sp. 1	US	GS01208869	GS017173	41.18900	-73.22200	Connecticut
R. sp. 1	US	GS01208990	GS017203	41.29400	-73.39600	Connecticut
R. sp. 1	US	GS01209700	GS017193	41.16400	-73.42000	Connecticut
R. sp. 1	US	GS01209710	GS100570	41.13528	-73.42667	Connecticut
R. sp. 1	US	GS01209901	GS017213	41.06600	-73.55000	Connecticut
R. lowei	US	GS012464770	GS001361	47.30280	-118.36810	Washington
R. sp. 2	US	GS013092747	GS167613	42.56250	-114.49390	Idaho
R. sp. 2	US	GS013092747	GS181541	42.56250	-114.49390	Idaho
R. sp. 1	US	GS0134273950	GS008363	42.96940	-74.96000	New York
R. sp. 1	US	GS0134907160	GS008763	42.84170	-74.70610	New York
R. sp. 1	US	GS0134909502	GS008712	42.84030	-74.61000	New York
R. sp. 1	US	GS01349100	GS008732	42.84670	-74.60330	New York
R. sp. 1	US	GS01349150	GS008752	42.87611	-74.60333	New York
R. sp. 1	US	GS01356190	GS008843	42.78330	-73.85750	New York
R. sp. 1	US	GS01357500	GS008900	42.78530	-73.70810	New York
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R. sp. 1	US	GS01361200	GS008003	42.21500	-73.72940	New York
R. sp. 3	US	GS01361200	GS008003	42.21500	-73.72940	New York
R. sp. 1	US	GS01362200	GS008530	42.11690	-74.38060	New York
R. sp. 1	US	GS01371500	GS008880	41.68610	-74.16560	New York
R. sp. 1	US	GS01372051	GS008503	41.71000	-73.92670	New York
R. sp. 1	US	GS01372200	GS008283	41.81530	-73.76390	New York
R. sp. 3	US	GS01372200	GS008283	41.81530	-73.76390	New York
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R. sp. 1	US	GS01390450	GS028173	41.05890	-74.09560	New Jersey
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R. sp. 1	US	GS01397295	GS028253	40.53780	-74.83780	New Jersey
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R. sp. 1	US	GS01400000	NJP024	40.56940	-74.67920	New Jersey
R. sp. 1	US	GS01401000	GS028303	40.33310	-74.68220	New Jersey
R. sp. 1	US	GS01401600	GS028313	40.41444	-74.65056	New Jersey
R. sp. 1	US	GS01403300	GS117832	40.55940	-74.52810	New Jersey
R. sp. 1	US	GS01403900	GS194093	40.58500	-74.50830	New Jersey
R. lowei	US	GS014205400	GSN99372	45.68083	-123.07000	Oregon
R. sp. 1	US	GS01443290	GS028353	41.09890	-74.69110	New Jersey
R. sp. 1	US	GS01443310	GS028363	41.12810	-74.72920	New Jersey
R. sp. 1	US	GS01443515	GS028373	40.98080	-74.96110	New Jersey
R. sp. 1	US	GS01454700	GSN00487	40.66920	-75.23670	Pennsylvania
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R. sp. 1	US	GS01614500	GS018883	39.71580	-77.82440	Maryland
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R. sp. 1	US	GS01621400	GS018253	38.43110	-78.88390	Virginia
R. sp. 1	US	GS01624490	GS018273	38.15080	-79.05940	Virginia
R. sp. 1	US	GS01636500	GS018474	39.28190	-77.78940	West Virginia
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R. sp. 1	US	GS0209517912	GS108598	36.08917	-79.82889	North Carolina
R. sp. 1	US	GS0209647295	GS108612	36.10889	-79.40333	North Carolina
R. sp. 1	US	GS02097464	GS108456	35.92361	-79.11556	North Carolina
R. sp. 1	US	GS02099238	GS108588	35.99806	-79.92667	North Carolina
R. sp. 1	US	GS02100634	GS108498	35.65905	-79.77698	North Carolina
R. sp. 1	US	GS02145112	GS024143	35.26030	-81.07390	North Carolina
R. sp. 1	US	GS02398300	GSN00879	34.29030	-85.50920	Alabama
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R. sp. 1	US	GS03178000	GSL00764	37.43330	-81.11110	West Virginia
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R. sp. 1	US	GS03474000	GSL00096	36.80720	-81.62220	Virginia
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R. sp. 3	US	GS05058700	GS019543	46.44690	-97.67890	North Dakota
R. sp. 1	US	GS05062500	GS019133	47.26670	-96.24440	Minnesota
R. sp. 3	US	GS05082625	GS019033	47.93190	-97.51420	North Dakota
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R. sp. 1	US	GS05267000	GSL00112	45.86110	-94.35830	Minnesota
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R. sp. 1	US	GS05551340	GSN20318	41.82220	-88.32470	Illinois
R. sp. 1	US	GS05551548	GSN21624	41.68610	-88.34940	Illinois
R. sp. 1	US	GS05551695	GSN20679	41.68220	-88.41360	Illinois
R. sp. 1	US	GS05552450	GSN20683	41.43670	-88.80390	Illinois

R. sp. 1	US	GS05572000	GSL00037	40.03080	-88.58890	Illinois
R. sp. 1	US	GS05575850	GSL00308	39.69610	-89.57250	Illinois
R. sp. 1	US	GS05583000	GSL00044	40.12390	-89.98500	Illinois
R. sp. 1	US	GS05584500	GSL00320	40.33030	-90.89610	Illinois
R. sp. 1	US	GS05586645	GSL00340	39.30440	-89.78750	Illinois
R. lowei	US	GS06191500	GSN59205	45.11190	-110.79360	Montana
R. sp. 2	US	GS06191500	GSN59205	45.11190	-110.79360	Montana
R. sp. 3	US	GS06191500	GSN59205	45.11190	-110.79360	Montana
R. lowei	US	GS06192500	GSN58747	45.59720	-110.56530	Montana
R. sp. 3	US	GS06192500	GSN58747	45.59720	-110.56530	Montana
R. lowei	US	GS06214500	GSN58753	45.80000	-108.46670	Montana
R. sp. 3	US	GS06214500	GSN58753	45.80000	-108.46670	Montana
R. sp. 3	US	GS06218000	GSN58755	46.14310	-107.55140	Montana
R. sp. 3	US	GS06295000	GSN58761	46.26610	-106.69000	Montana
R. sp. 3	US	GS06309000	GSN58765	46.42170	-105.86060	Montana
R. sp. 3	US	GS06713500	GS007313	39.74250	-104.99940	Colorado
R. sp. 3	US	GS06714000	GSN94057	39.75970	-105.00280	Colorado
R. sp. 3	US	GS06741510	GS007173	40.37860	-105.06060	Colorado
R. sp. 3	US	GS06752280	GS007223	40.55190	-105.01080	Colorado
R. sp. 3	US	GS06753990	GS135378	40.44250	-104.58830	Colorado
R. sp. 3	US	GS06765500	GS007041	41.11810	-100.77280	Nebraska
R. sp. 1	US	GS06775900	GS198786	41.77861	-100.52528	Nebraska
R. sp. 1	US	GS06919925	GS021123	37.83470	-93.67280	Missouri
R. sp. 1	US	GS06923250	GS021154	37.68420	-92.92420	Missouri
R. sp. 1	US	GS07053250	GS021281	36.45444	-93.35611	Arkansas
R. sp. 1	US	GS07053250	GS171574	36.45444	-93.35611	Arkansas
R. sp. 1	US	GS08050800	GS002003	33.55440	-96.94690	Texas
R. sp. 1	US	GS08057410	GS002351	32.70720	-96.73560	Texas
R. sp. 3	US	GS08062500	GS002371	32.42640	-96.46280	Texas
R. sp. 1	US	GS08227000	GS010131	38.16330	-106.29000	Colorado
R. sp. 2	US	GS08276500	GS010093	36.32000	-105.75390	New Mexico
R. sp. 4	US	GS08276500	GS010093	36.32000	-105.75390	New Mexico
R. sp. 4	US	GS08290000	GS010063	36.07390	-106.11110	New Mexico
R. sp. 2	US	GS08313350	GS010353	35.77640	-106.26830	New Mexico
R. sp. 4	US	GS08331000	GS010423	34.90580	-106.68440	New Mexico
R. sp. 3	US	GS09149480	GS025131	38.64580	-108.04830	Colorado
R. sp. 3	US	GS09163500	GS114046	39.13280	-109.02640	Colorado
R. californica	US	GS09505800	GS145584	34.53860	-111.69330	Arizona
R. sp. 4	US	GS09508500	GSL00802	34.07310	-111.71560	Arizona
R. sp. 3	US	GS10038000	GS111770	42.12670	-110.97250	Wyoming
R. sp. 3	US	GS10102200	GSN80914	41.92640	-111.85280	Utah
R. sp. 2	US	GS10130500	GSN24747	40.89530	-111.40110	Utah
R. sp. 2	US	GS10168000	GSN24106	40.66390	-111.90110	Utah
R. sp. 3	US	GS10168000	GSN24106	40.66390	-111.90110	Utah
R. sp. 3	US	GS10172200	GS111847	40.78000	-111.80530	Utah
R. lowei	US	GS10309010	GS000001	38.87830	-119.68830	Nevada

R. sp. 2	US	GS10309010	GS000001	38.87830	-119.68830	Nevada
R. lowei	US	GS10309500	GS000323	38.77690	-119.89830	California
R. sp. 2	US	GS10309500	GS000323	38.77690	-119.89830	California
R. californica	US	GS10310200	GS000503	38.80890	-119.77610	California
R. lowei	US	GS10310358	GS000313	38.97110	-119.81670	Nevada
R. sp. 2	US	GS10310358	GS000313	38.97110	-119.81670	Nevada
R. sp. 3	US	GS10311400	GS000293	39.18110	-119.69440	Nevada
R. lowei	US	GS10311700	GS000283	39.23780	-119.58720	Nevada
R. sp. 2	US	GS10311700	GS000283	39.23780	-119.58720	Nevada
R. californica	US	GS10312000	GS000133	39.29310	-119.25060	Nevada
R. sp. 5	US	GS10346000	GS000463	39.42810	-120.03310	California
R. sp. 2	US	GS10347705	GS000433	39.52310	-119.83170	Nevada
R. sp. 2	US	GS10348200	GS000453	39.51970	-119.74080	Nevada
R. californica	US	GS10350050	GS000243	39.51000	-119.64780	Nevada
R. sp. 3	US	GS10350050	GS000243	39.51000	-119.64780	Nevada
R. lowei	US	GS10350500	GS000633	39.56530	-119.48390	Nevada
R. sp. 2	US	GS10350500	GS000633	39.56530	-119.48390	Nevada
R. sp. 5	US	GS10350500	GS000633	39.56530	-119.48390	Nevada
R. sp. 3	US	GS10351650	GS000573	39.63220	-119.28220	Nevada
R. lowei	US	GS10351690	GS000403	39.73720	-119.32330	Nevada
R. sp. 2	US	GS10351690	GS000403	39.73720	-119.32330	Nevada
R. sp. 5	US	GS11074000	GS133940	33.88330	-117.64440	California
R. californica	US	GS11367808	GS029013	41.09420	-122.11560	California
R. lowei	US	GS11367808	GS029013	41.09420	-122.11560	California
R. lowei	US	GS11383500	GS029033	40.01420	-121.94720	California
R. californica	US	GS11384200	GS029073	39.72720	-121.86220	California
R. californica	US	GS11447360	GS137234	38.64190	-121.38170	California
R. sp. 2	US	GS12103380	GS030193	47.18190	-121.38750	Washington
R. sp. 2	US	GS12103395	GS030043	47.20580	-121.40470	Washington
R. sp. 2	US	GS12108500	GS030203	47.27580	-122.05830	Washington
R. sp. 2	US	GS12112600	GS030213	47.31250	-122.16420	Washington
R. sp. 2	US	GS12128000	GS030393	47.69580	-122.27500	Washington
R. lowei	US	GS12212100	GS030173	48.92670	-122.49500	Washington
R. lowei	US	GS12462640	GS120732	47.29333	-120.15361	Washington
R. sp. 2	US	GS12471400	GS120479	47.01030	-119.13610	Washington
R. lowei	US	GS12472000	GS001153	46.91940	-119.23780	Washington
R. sp. 2	US	GS12483940	GS120119	47.01639	-120.47500	Washington
R. sp. 2	US	GS12500420	GSN63853	46.54610	-120.43690	Washington
R. lowei	US	GS12502500	GS119512	46.53610	-120.47220	Washington
R. lowei	US	GS12508820	GS119791	46.28944	-119.97833	Washington
R. lowei	US	GS12509492	GSN63842	46.21250	-119.77780	Washington
R. sp. 2	US	GS12509492	GSN63842	46.21250	-119.77780	Washington
R. lowei	US	GS12509710	GSN63884	46.23330	-119.67720	Washington
R. lowei	US	GS12510500	GS151671	46.25361	-119.47694	Washington
R. lowei	US	GS13010065	GS009453	44.08920	-110.69390	Wyoming
R. sp. 2	US	GS13010065	GS009453	44.08920	-110.69390	Wyoming
R. lowei	US	GS13027500	GS009103	43.07970	-111.03670	Wyoming
R. sp. 2	US	GS13044550	GS009213	44.12810	-111.17360	Idaho

R. lowei	US	GS13081500	GS009051	42.67310	-113.49940	Idaho
R. lowei	US	GS13091995	GS167625	42.32470	-114.27220	Idaho
R. lowei	US	GS13094000	GS009043	42.66610	-114.71140	Idaho
R. lowei	US	GS13107200	GS009013	42.45280	-114.86110	Idaho
R. sp. 2	US	GS13107200	GS009013	42.45280	-114.86110	Idaho
R. sp. 2	US	GS13120500	GS009423	43.99830	-114.02000	Idaho
R. sp. 2	US	GS13152500	GS009151	42.88670	-114.80220	Idaho
R. lowei	US	GS13154500	GS009023	43.00220	-115.20170	Idaho
R. sp. 2	US	GS13154500	GS009023	43.00220	-115.20170	Idaho
R. lowei	US	GS13346000	GS001081	46.92080	-117.31780	Washington
R. sp. 3	US	GS13346990	GS001013	46.72110	-117.13610	Washington
R. sp. 3	US	GS13349200	GS001401	46.87560	-117.34500	Washington
R. lowei	US	GS14201300	GS016023	45.10060	-122.82060	Oregon
R. lowei	US	GS14203750	GS016403	45.64360	-123.36920	Oregon
R. sp. 2	US	GS14203750	GS016403	45.64360	-123.36920	Oregon
R. lowei	US	GS14206435	GS145387	45.52067	-122.89955	Oregon
R. sp. 2	US	GS14206435	GS145387	45.52067	-122.89955	Oregon
R. lowei	US	GS14206950	GS180413	45.40361	-122.75361	Oregon
R. lowei	US	GS14207500	GS016063	45.35080	-122.67500	Oregon
R. californica	US	GS384942122105601	GS029263	38.82830	-122.18220	California
R. sp. 5	US	GS384942122105601	GS029263	38.82830	-122.18220	California
R. sp. 1	US	GS385234087071801	GSL0W173	38.87610	-87.12170	Indiana
R. sp. 3	US	GS391732085414401	GSL0W177	39.29220	-85.69560	Indiana
R. sp. 1	US	GS392400083494000	GSN82509	39.40000	-83.82780	Ohio
R. sp. 3	US	GS392400083494000	GSN82509	39.40000	-83.82780	Ohio
R. sp. 3	US	GS393259085101200	GSN00323	39.54970	-85.17000	Indiana
R. sp. 3	US	GS393306086585201	GSL0W179	39.55170	-86.98110	Indiana
R. sp. 3	US	GS393554105573001	GS007193	39.59830	-105.95830	Colorado
R. sp. 1	US	GS393557105033101	GS110931	39.59917	-105.05861	Colorado
R. sp. 3	US	GS393557105033101	GS110931	39.59917	-105.05861	Colorado
R. sp. 1	US	GS393613104511401	GS111138	39.60361	-104.85389	Colorado
R. sp. 3	US	GS393613104511401	GS111138	39.60361	-104.85389	Colorado
R. sp. 1	US	GS393814084043500	GSN82595	39.63740	-84.07640	Ohio
R. sp. 3	US	GS393814084043500	GSN82595	39.63740	-84.07640	Ohio
R. sp. 1	US	GS393837083505401	GS141632	39.64388	-83.84860	Ohio
R. sp. 1	US	GS393903084110500	GSN82569	39.65100	-84.18480	Ohio
R. sp. 3	US	GS393903084110500	GSN82569	39.65100	-84.18480	Ohio
R. sp. 3	US	GS393944084120700	GSN55367	39.66220	-84.20190	Ohio
R. sp. 1	US	GS394111084234200	GSN82599	39.68640	-84.39500	Ohio
R. sp. 1	US	GS394253083583300	GSN82620	39.71470	-83.97580	Ohio
R. sp. 1	US	GS394510084384100	GSN82700	39.75280	-84.64470	Ohio
R. sp. 1	US	GS394727083523000	GSN82823	39.79080	-83.87500	Ohio
R. sp. 3	US	GS394953084244100	GSN82813	39.83140	-84.41140	Ohio
R. sp. 1	US	GS395327085190801	GS141456	39.89085	-85.31892	Indiana
R. sp. 3	US	GS395327085190801	GS141456	39.89085	-85.31892	Indiana
R. californica	US	GS395336121413201	GS029313	39.89330	-121.69220	California
R. lowei	US	GS395336121413201	GS029313	39.89330	-121.69220	California
R. sp. 1	US	GS395534084091400	GSN00335	39.92610	-84.15390	Ohio
R. sp. 3	US	GS395534084091400	GSN00335	39.92610	-84.15390	Ohio
R. sp. 1	US	GS395554105085601	GS111025	39.93167	-105.14889	Colorado
R. sp. 3	US	GS395554105085601	GS111025	39.93167	-105.14889	Colorado

R. sp. 1	US	GS395912084214000	GSN82489	39.98680	-84.36110	Ohio
R. sp. 3	US	GS395912084214000	GSN82489	39.98680	-84.36110	Ohio
R. sp. 1	US	GS400134084400300	GSN82658	40.02610	-84.66750	Ohio
R. sp. 1	US	GS400925105023201	GS110992	40.15694	-105.04222	Colorado
R. sp. 3	US	GS400925105023201	GS110992	40.15694	-105.04222	Colorado
R. sp. 3	US	GS400927111354501	GSN24735	40.15750	-111.59580	Utah
R. lowei	US	GS400959111363201	GSN23649	40.16640	-111.60890	Utah
R. sp. 3	US	GS400959111363201	GSN23649	40.16640	-111.60890	Utah
R. lowei	US	GS401442111402201	GSN24707	40.24500	-111.67280	Utah
R. sp. 3	US	GS401442111402201	GSN24707	40.24500	-111.67280	Utah
R. sp. 2	US	GS401653111400301	GSN24737	40.28140	-111.66750	Utah
R. sp. 3	US	GS401653111400301	GSN24737	40.28140	-111.66750	Utah
R. sp. 3	US	GS401850111392201	GSN24733	40.31390	-111.65610	Utah
R. sp. 1	US	GS402108076363701	GS011183	40.35220	-76.61030	Pennsylvania
R. sp. 3	US	GS402340104575101	GS007161	40.39440	-104.96420	Colorado
R. sp. 1	US	GS402549078213001	GS011103	40.39530	-78.40810	Pennsylvania
R. sp. 3	US	GS403048105042701	GS111104	40.51333	-105.07439	Colorado
R. sp. 3	US	GS403308105001601	GS116019	40.55222	-105.00444	Colorado
R. sp. 1	US	GS403356105024001	GS111000	40.56556	-105.04444	Colorado
R. sp. 3	US	GS403356105024001	GS111000	40.56556	-105.04444	Colorado
R. sp. 1	US	GS403936078152101	GS011093	40.66000	-78.25580	Pennsylvania
R. lowei	US	GS403945111501001	GSN23866	40.66250	-111.83610	Utah
R. sp. 3	US	GS403945111501001	GSN23866	40.66250	-111.83610	Utah
R. lowei	US	GS404000111515801	GSN23870	40.66670	-111.86610	Utah
R. sp. 3	US	GS404000111515801	GSN23870	40.66670	-111.86610	Utah
R. sp. 3	US	GS404140111481601	GSN24081	40.69440	-111.80440	Utah
R. sp. 3	US	GS404318111310401	GSN23886	40.72167	-111.51778	Utah
R. sp. 1	US	GS404502111220801	GSN24717	40.75060	-111.36890	Utah
R. sp. 3	US	GS404519111334801	GSN24749	40.75530	-111.56330	Utah
R. sp. 3	US	GS404609111345901	GSN24181	40.76920	-111.58310	Utah
R. sp. 1	US	GS404621077050901	GS011213	40.77250	-77.08580	Pennsylvania
R. sp. 3	US	GS405733102230201	GS007253	40.95920	-102.38390	Colorado
R. sp. 1	US	GS405854111534801	GSN23874	40.98170	-111.89670	Utah
R. lowei	US	GS410041111581101	GSN23814	41.01140	-111.96970	Utah
R. sp. 3	US	GS410041111581101	GSN23814	41.01140	-111.96970	Utah
R. lowei	US	GS410250111571501	GSN23819	41.04720	-111.95420	Utah
R. sp. 3	US	GS410250111571501	GSN23819	41.04720	-111.95420	Utah
R. sp. 3	US	GS410342111574201	GSN24731	41.06170	-111.96170	Utah
R. sp. 3	US	GS410453111570001	GSN23906	41.08140	-111.95000	Utah
R. sp. 1	US	GS410714104480101	GS111098	41.12056	-104.80028	Wyoming
R. lowei	US	GS411407111580501	GSN23800	41.23530	-111.96810	Utah
R. sp. 3	US	GS411407111580501	GSN23800	41.23530	-111.96810	Utah
R. lowei	US	GS411413111554601	GSN24745	41.23690	-111.92940	Utah
R. sp. 2	US	GS411413111554601	GSN24745	41.23690	-111.92940	Utah
R. sp. 3	US	GS411413111554601	GSN24745	41.23690	-111.92940	Utah
R. lowei	US	GS411413111564101	GSN24741	41.23690	-111.94470	Utah
R. sp. 2	US	GS411413111564101	GSN24741	41.23690	-111.94470	Utah
R. sp. 3	US	GS411413111564101	GSN24741	41.23690	-111.94470	Utah
R. sp. 1	US	GS412829097405601	GS112206	41.47475	-97.68261	Nebraska
R. sp. 1	US	GS413311097171001	GS112384	41.55312	-97.28652	Nebraska
R. sp. 1	US	GS413850099402301	GS112673	41.64715	-99.67341	Nebraska

R. lowei	US	GS441430123054803	GS016133	44.24170	-123.09670	Oregon
R. lowei	US	GS441549123232503	GS016081	44.26360	-123.39030	Oregon
R. lowei	US	GS443138123120901	GS016143	44.52720	-123.20250	Oregon
R. sp. 2	US	GS443138123120901	GS016143	44.52720	-123.20250	Oregon
R. lowei	US	GS444002123163603	GS016153	44.66720	-123.27670	Oregon
R. lowei	US	GS445551123015800	GS145513	44.93068	-123.03398	Oregon
R. sp. 2	US	GS445551123015800	GS145513	44.93068	-123.03398	Oregon
R. lowei	US	GS450022123012400	GS145405	45.00595	-123.02454	Oregon
R. sp. 2	US	GS450022123012400	GS145405	45.00595	-123.02454	Oregon
R. lowei	US	GS451138122431702	GS016163	45.19390	-122.72140	Oregon
R. lowei	US	GS451259122481902	GS016253	45.21640	-122.80530	Oregon
R. lowei	US	GS451350122415603	GS016263	45.23060	-122.69890	Oregon
R. lowei	US	GS452526122364400	GS145479	45.42373	-122.61343	Oregon
R. sp. 2	US	GS452526122364400	GS145479	45.42373	-122.61343	Oregon
R. lowei	US	GS453205122223701	GS016273	45.53470	-122.37690	Oregon
R. sp. 2	US	GS453205122223701	GS016273	45.53470	-122.37690	Oregon
R. lowei	US	GS454510122424900	GS145561	45.75262	-122.71482	Washington
R. lowei	US	GS455122122310600	GS145527	45.85595	-122.51954	Washington
R. lowei	US	GS461315119452400	GSN63846	46.22090	-119.75680	Washington
R. sp. 2	US	GS461315119452400	GSN63846	46.22090	-119.75680	Washington
R. lowei	US	GS461517119402500	GS119810	46.25480	-119.67360	Washington
R. lowei	US	GS462018120012000	GSN63834	46.33840	-120.02210	Washington
R. lowei	US	GS462023120075200	GS122292	46.33947	-120.13214	Washington
R. sp. 2	US	GS463147120455700	GSN63900	46.52970	-120.76580	Washington
R. sp. 3	US	GS464539117133000	GS001111	46.76080	-117.22500	Washington
R. lowei	US	GS465537116422500	GS001073	46.92690	-116.70690	Idaho
R. sp. 2	US	GS465537116422500	GS001073	46.92690	-116.70690	Idaho
R. sp. 2	US	GS465637116381400	GS001333	46.94360	-116.63720	Idaho
R. sp. 1	US	GS473130096155001	GS019273	47.52500	-96.26390	Minnesota
R. californica	SCB	GSSJ1	UCOB_2483	34.45617	-119.81107	California
R. californica	SCB	LADC1	UCOB_2485	34.15568	-118.63242	California
R. sp. 5	SCB	MAMD1	UCOB_2683	34.11625	-118.75612	California
R. sp. 5	SCB	MCMC1	UCOB_2685	34.44220	-119.71101	California
R. californica	SCB	SASA4	UCOB_2837	34.16927	-116.82033	California
R. californica	SCB	SCSF1	UCOB_2710	34.31409	-118.31481	California
R. sp. 5	SCB	SCSF1	UCOB_2710	34.31409	-118.31481	California
R. sp. 5	SCB	SCSP2	UCOB_2714	34.44469	-118.92741	California
R. stoermeri	SCB	SGBC1	UCOB_2527	34.24154	-117.88599	California
R. californica	SCB	SJBL1	UCOB_2732	33.63432	-117.55474	California
R. sp. 5	SCB	SJOS1	UCOB_2552	33.53918	-117.67555	California
R. californica	SCB	SJTC2	UCOB_2557	33.67420	-117.53048	California
R. sp. 5	SCB	SJTC2	UCOB_2557	33.67420	-117.53048	California
R. californica	SCB	SJTC3	UCOB_2559	33.53643	-117.66497	California
R. sp. 5	SCB	SJTC3	UCOB_2559	33.53643	-117.66497	California
R. californica	SCB	SMAD1	UCOB_2638	33.51272	-117.27038	California
R. sp. 5	SCB	SRSD2	UCOB_2760	32.83945	-117.04469	California
R. sp. 5	SCB	SYHC1	UCOB_2589	34.58740	-119.98656	California

Taxon	Algal Sample ID	pH	Phosphorus	Silica	Conductivity	Sulfate	Abun-dance	Sample Date
R. californica	UCOB_6154	8.60	0.01270	12.400	151.000	3.85	19	8/9/2010
R. lowei	UCOB_6154	8.60	0.01270	12.400	151.000	3.85	38	8/9/2010
R. californica	UCOB_6302	8.10	0.02520	14.700	78.200	1.61	58	9/14/2010
R. californica	UCOB_3859	8.20	0.04200	34.100	494.000	12.9	79	8/24/2009
R. californica	UCOB_3895	8.20	0.04200	34.100	494.000	12.9	101	9/15/2009
R. californica	UCOB_3915	6.70	0.01810	10.700	78.300	3.7	59	8/18/2009
R. californica	UCOB_3084	8.50	0.13000	42.300	101.000	4.26	14	8/26/2008
R. californica	UCOB_7629	8.28	0.02100	11.300	148.000	6.29	57	8/30/2011
R. californica	UCOB_7331	7.98	0.01220	18.700	88.000	1.875	52	8/2/2011
R. stoermeri	UCOB_9232	8.75	0.00770	21.700	162.800	2.23	2	8/8/2012
R. californica	UCOB_9233	8.84	0.02990	29.200	121.200	2.06	40	8/27/2012
R. californica	UCOB_7738a	7.98	0.02200	13.600	154.000	7.28	72	7/26/2011
R. californica	UCOB_6165	7.60	0.03355	11.300	181.000	8.59	30	7/21/2010
R. lowei	UCOB_6165	7.60	0.03355	11.300	181.000	8.59	44	7/21/2010
R. californica	UCOB_7337	7.96	0.02610	17.000	230.000	14.6	40	7/27/2011
R. lowei	UCOB_7337	7.96	0.02610	17.000	230.000	14.6	75	7/27/2011
R. californica	UCOB_6368						33	5/11/2010
R. californica	UCOB_6369						42	6/15/2010
R. californica	UCOB_7248	7.86	0.10300	17.000	169.700	2.675	78	7/13/2011
R. californica	UCOB_3092	7.83	0.03730	12.800	219.900	2.62	103	9/9/2008
R. stoermeri	UCOB_3092	7.83	0.03730	12.800	219.900	2.62	60	9/9/2008
R. californica	UCOB_3077	8.00	0.20300	22.400	262.000	11.1	102	8/18/2008
R. californica	UCOB_3989	8.80	0.02580	12.100	111.900	1.64	15	8/27/2009
R. stoermeri	UCOB_3903	8.80	0.02580	12.100	111.900	1.64	84	8/27/2009
R. californica	UCOB_3897	8.50	0.02950	14.200	277.500	3.45	38	6/24/2009
R. californica	UCOB_7327	8.11	0.01300	12.600	95.700	7.03	31	7/25/2011
R. californica	UCOB_6168	7.70	0.00950	13.900	278.000	8.93	243	8/17/2010
R. lowei	UCOB_6042						78	10/4/2010
R. lowei	UCOB_6041						39	10/5/2010
R. lowei	UCOB_5956						41	9/15/2010
R. lowei	UCOB_6040						177	10/3/2010
R. californica	UCOB_7241	7.97	0.02125	10.400	185.000	21.8	58	7/12/2011
R. californica	UCOB_3462						117	6/8/2009
R. californica	UCOB_7683	7.85	0.04620	26.500	133.000	4.825	50	8/22/2011
R. californica	UCOB_4843						42	6/30/2009
R. californica	UCOB_5646	7.56	0.05030	24.850	161.000	6.64	75	7/26/2010
R. californica	UCOB_5708	7.91	0.06170	35.650	148.000	6.485	107	8/31/2010
R. californica	UCOB_5751	8.07	0.04430	28.900	170.000	7.75	14	6/28/2010
R. lowei	UCOB_5751	8.07	0.04430	28.900	170.000	7.75	126	6/28/2010
R. californica	UCOB_6170	7.09	0.03820	17.700	178.900	4.11	63	9/15/2010
R. stoermeri	UCOB_9230	8.69	0.00530	15.700	269.800	6.81	84	7/31/2012
R. stoermeri	UCOB_2979	8.71	0.01060	14.400	467.000	2	12	8/20/2008
R. californica	UCOB_8736						192	6/20/2012
R. californica	UCOB_4292	8.22	0.07130	30.900	319.000	6.75	38	7/15/2009
R. californica	UCOB_3998	7.38	0.08080	30.400	400.000	6.45	82	7/14/2009
R. californica	UCOB_6313	7.54	0.03820	23.900	348.100	34.7	39	6/29/2010
R. californica	UCOB_7117	8.37	0.01450	22.450	263.700	26.5	118	6/14/2011
R. californica	UCOB_4290	7.40	0.07910	26.900	312.000	35.5	93	6/16/2009
R. californica	UCOB_2941						63	6/18/2008

R. californica	UCOB_3971	6.95	0.03935	16.400	326.000	18.45	82	6/15/2009
R. lowei	UCOB_8630						46	5/22/2012
R. californica	UCOB_6173	9.04	0.02470	7.070	740.000	55.3	57	7/14/2010
R. californica	UCOB_8729						46	6/5/2012
R. californica	UCOB_6315	8.29	0.01300	16.200	394.400	23.9	120	7/15/2010
R. californica	UCOB_8631						162	5/23/2012
R. californica	UCOB_3983	8.00	0.05170	13.600	123.700	40.2	93	6/17/2009
R. californica	UCOB_8735						76	6/19/2012
R. californica	UCOB_3987	8.40	0.06700	19.900	151.700	41.3	58	7/13/2009
R. californica	UCOB_8731						16	6/11/2012
R. lowei	UCOB_8731						50	6/11/2012
R. californica	UCOB_8740						33	6/27/2012
R. californica	UCOB_8727						98	5/29/2012
R. californica	UCOB_8716						85	8/23/2011
R. californica	UCOB_8718						3	8/25/2011
R. lowei	UCOB_8718						71	8/25/2011
R. californica	UCOB_8721						73	8/31/2011
R. californica	UCOB_8737						42	6/21/2012
R. californica	UCOB_8715						13	8/16/2011
R. lowei	UCOB_8715						46	8/16/2011
R. californica	UCOB_8726						16	9/14/2011
R. lowei	UCOB_8726						34	9/14/2011
R. californica	UCOB_8724						2	9/12/2011
R. lowei	UCOB_8724						47	9/12/2011
R. californica	UCOB_8722						9	9/6/2011
R. lowei	UCOB_8722						38	9/6/2011
R. lowei	UCOB_8723						32	9/7/2011
R. californica	UCOB_8720						58	8/30/2011
R. californica	UCOB_8713						75	8/10/2011
R. californica	UCOB_8709						91	8/1/2011
R. californica	UCOB_7118	7.74	0.05100	43.100	100.000	1.64	190	6/13/2011
R. lowei	UCOB_7109	8.57	0.02800	17.800	1016.000	167	54	6/1/2011
R. californica	UCOB_3027	8.43	0.28900	41.100	561.000	66.9	60	6/4/2008
R. californica	UCOB_3033						22	6/17/2008
R. californica	UCOB_3029	8.30	0.17600	23.000	388.000	26.45	34	6/4/2008
R. californica	UCOB_6318	7.44	0.05875	28.700	261.500	40.15	11	6/28/2010
R. californica	UCOB_6319	7.67	0.06560	28.200	303.900	27.1	69	6/29/2010
R. californica	UCOB_2966	8.10	0.03280	26.200	1260.000	76.2	41	7/16/2008
R. californica	UCOB_6320	7.90	0.01470	25.000	444.100	26.55	51	6/30/2010
R. sp. 5	UCOB_3032	8.17	0.11500	14.700	1640.000	275	13	6/17/2008
R. californica	UCOB_3957	8.20	0.56000	14.900	178.900	197	52	6/16/2009
R. stoermeri	UCOB_3964	8.10	0.03850	15.900	405.000	15.9	69	6/17/2009
R. californica	UCOB_6321	7.70	0.01870	21.000	342.200	28	38	6/29/2010
R. californica	UCOB_6322	8.00	0.01080	18.500	364.700	25.7	137	6/29/2010
R. californica	UCOB_6323	7.90	0.01880	28.300	267.000	25.5	134	6/22/2010
R. californica	UCOB_6324	7.20	0.03860	30.050	159.000	2.54	76	6/21/2010
R. californica	UCOB_7121	8.45	0.00610	20.600	322.000	14.2	15	6/14/2011
R. californica	UCOB_6325	8.52	0.01830	13.400	261.200	17.4	13	7/13/2010
R. californica	UCOB_7120	8.06	0.01320	28.700	172.000	7.59	119	6/13/2011
R. californica	UCOB_7782						134	7/18/2011
R. californica	UCOB_6326	8.46	0.02000	20.700	323.100	11	10	6/23/2010

R. californica	UCOB_6327	8.16	0.01710	17.700	301.400	19.5	185	6/22/2010
R. californica	UCOB_6328	8.50	0.00950	18.300	372.200	20.6	5	6/23/2010
R. stoermeri	UCOB_6328	8.50	0.00950	18.300	372.200	20.6	15	6/23/2010
R. californica	UCOB_6329	7.88	0.05770	27.000	303.700	6.83	34	6/21/2010
R. californica	UCOB_6331	7.50	0.01000	18.300	271.000	32.8	44	6/23/2010
R. californica	UCOB_7104	7.46	0.02260	27.800	337.300	36.6	33	6/16/2011
R. californica	UCOB_7105	7.46	0.02260	27.800	337.300	36.6	11	6/16/2011
R. californica	UCOB_6332	7.90	0.02220	36.200	282.000	25.1	30	6/22/2010
R. californica	UCOB_6333	7.44	0.47700	28.200	780.000	89.1	9	6/24/2010
R. californica	UCOB_3920	7.80	0.24300	21.700	153.700	88.6	9	6/9/2009
R. californica	UCOB_6334	8.07	0.22800	25.300	748.000	95.7	9	6/15/2010
R. californica	UCOB_7122	8.31	0.00940	21.700	629.000	60	58	6/15/2011
R. californica	UCOB_7780						26	8/11/2011
R. sp. 5	UCOB_7122	8.31	0.00940	21.700	629.000	60	58	6/15/2011
R. sp. 5	UCOB_7780						26	8/11/2011
R. californica	UCOB_6336	8.10	0.01240	21.100	517.000	159	11	6/16/2010
R. sp. 5	UCOB_6339	8.10	0.00750	16.700	499.000	68.2	79	6/15/2010
R. sp. 5	UCOB_3782	8.19	0.02510	27.000	1080.000	169	25	6/11/2009
R. sp. 5	UCOB_3786	8.42	0.00885	7.750	877.000	244	55	5/20/2009
R. sp. 5	UCOB_5847	7.77	0.02355	15.000	1742.000	555	31	6/10/2010
R. sp. 5	UCOB_7203	7.69	0.04930	13.400	686.000	84.2	87	6/13/2011
R. californica	UCOB_7201	8.41	0.02870	18.000	385.000	11.5	45	7/12/2011
R. sp. 5	UCOB_7207	8.21	0.04160	14.300	832.000	224	16	6/14/2011
R. californica	UCOB_2920						94	6/18/2008
R. californica	UCOB_2922						24	6/5/2008
R. californica	UCOB_2923						113	6/4/2008
R. californica	UCOB_2927						45	6/4/2008
R. californica	UCOB_2928						75	6/19/2008
R. californica	UCOB_2929						17	6/10/2008
R. sp. 5	UCOB_3914	8.00	0.02670	20.200	169.900	324	29	6/4/2009
R. sp. 5	UCOB_5859	7.41	0.07420	31.300	1355.000	260	26	6/22/2010
R. sp. 5	UCOB_3760	7.38	0.23900	31.000	3471.000	1370	30	5/12/2009
R. californica	UCOB_3754	7.81	0.15200	46.200	2094.000	912	37	5/13/2009
R. sp. 5	UCOB_3779	7.70	0.16150	35.800	3692.000	1460	59	5/12/2009
R. sp. 5	UCOB_3785	7.81	0.02000	12.500	1438.000	411	25	5/14/2009
R. sp. 5	UCOB_3778	7.49	0.10400	22.600	1335.000	288	31	5/13/2009
R. californica	UCOB_7200	7.46	0.11500	34.000	1954.000	1560	28	6/8/2011
R. californica	UCOB_7206	7.69	0.08960	44.000	3548.000	1560	21	6/15/2011
R. sp. 5	UCOB_7206	7.69	0.08960	44.000	3548.000	1560	21	6/15/2011
R. sp. 5	UCOB_7197	7.51	0.07510	23.700	2945.000	1040	89	6/9/2011
R. sp. 5	UCOB_3788	7.15	0.19100	29.000	3116.000	1080	100	5/18/2009
R. californica	UCOB_3757	7.71	0.51200	40.600	4028.000	226	60	5/20/2009
R. sp. 5	UCOB_3757	7.71	0.51200	40.600	4028.000	226	153	5/20/2009
R. sp. 5	UCOB_7205	7.90	0.14400	34.100	3946.000	1620	117	6/15/2011
R. sp. 5	UCOB_8782	7.85	0.13200	28.500	2917.000	1100	47	6/18/2012
R. sp. 5	UCOB_8780	8.10	0.04810	30.400	3704.000	1410	45	6/19/2012
R. californica	UCOB_8787	7.66	0.16300	33.100	2832.000	886	29	6/21/2012
R. sp. 5	UCOB_8787	7.66	0.16300	33.100	2832.000	886	29	6/21/2012
R. californica	UCOB_2935						25	5/7/2008
R. sp. 5	UCOB_3014	9.06	0.20200	18.500	560.000	85.4	34	5/20/2008
R. californica	UCOB_3046	8.66	0.03770	31.200	220.500	3.93	235	6/30/2008

R. lowei	UCOB_3046	8.66	0.03770	31.200	220.500	3.93	235	6/30/2008
R. californica	UCOB_3049	8.81	0.04630	21.200	139.000	9.39	45	7/2/2008
R. californica	UCOB_3940						69	7/28/2009
R. californica	UCOB_6182						57	9/14/2010
R. californica	UCOB_3974	8.80	0.09440	43.300	78.700	2.72	5	6/29/2009
R. lowei	UCOB_3974	8.80	0.09440	43.300	78.700	2.72	62	6/29/2009
R. californica	UCOB_7237	8.20	0.02000	31.800	161.000	1.57	85	7/13/2011
R. californica	UCOB_7727	8.30	0.06440	50.400	131.700	1.74	20	9/8/2011
R. lowei	UCOB_7727	8.30	0.06440	50.400	131.700	1.74	205	9/8/2011
R. californica	UCOB_3004	8.25	0.05270	45.900	141.000	0.57	48	9/16/2008
R. californica	UCOB_3916						87	6/10/2009
R. lowei	UCOB_3916						87	6/10/2009
R. californica	UCOB_7632	8.40	0.05180	44.100	100.000	0.55	251	8/31/2011
R. lowei	UCOB_2957	8.17	0.06620	57.000	234.000	1.98	133	7/7/2008
R. californica	UCOB_2981	8.29	0.03170	38.200	137.500	0.92	9	8/21/2008
R. lowei	UCOB_2981	8.29	0.03170	38.200	137.500	0.92	19	8/21/2008
R. californica	UCOB_7631	8.33	0.02970	38.600	102.000	0.4	112	8/29/2011
R. californica	UCOB_2971	8.22	0.06510	55.200	294.000	2.25	9	7/30/2008
R. californica	UCOB_2991						7	9/10/2008
R. lowei	UCOB_2971	8.22	0.06510	55.200	294.000	2.25	9	7/30/2008
R. lowei	UCOB_2991						7	9/10/2008
R. californica	UCOB_2986	8.53	0.07950	63.600	221.800	0.84	6	9/3/2008
R. lowei	UCOB_2965						165	7/15/2008
R. lowei	UCOB_2986	8.53	0.07950	63.600	221.800	0.84	118	9/3/2008
R. californica	UCOB_7732	8.00	0.05990	40.000	89.600	21.25	73	9/7/2011
R. californica	UCOB_6186						132	7/26/2010
R. californica	UCOB_3041	8.30	0.03070	24.600	353.000	14.9	1	6/25/2008
R. californica	UCOB_3069						64	8/7/2008
R. californica	UCOB_6189						68	9/1/2010
R. stoermeri	UCOB_3041	8.30	0.03070	24.600	353.000	14.9	77	6/25/2008
R. stoermeri	UCOB_3069						15	8/7/2008
R. stoermeri	UCOB_6189						68	9/1/2010
R. lowei	UCOB_2990	8.31	0.01970	46.500	1142.000	3.32	67	9/10/2008
R. californica	UCOB_7729	8.20	0.02180	14.000	72.700	4.54	42	9/21/2011
R. californica	UCOB_4287	7.50	0.05810	24.200	92.000	2.12	75	7/1/2009
R. californica	UCOB_3866	7.90	0.01970	27.200	218.300	5.16	71	7/21/2009
R. californica	UCOB_7630	8.09	0.00750	20.200	146.600	4.01	144	8/17/2011
R. lowei	UCOB_7630	8.09	0.00750	20.200	146.600	4.01	54	8/17/2011
R. californica	UCOB_7624	8.08	0.01430	25.000	123.400	0.77	129	8/17/2011
R. lowei	UCOB_7624	8.08	0.01430	25.000	123.400	0.77	32	8/17/2011
R. californica	UCOB_2959	8.03	0.13500	23.000	151.500	3.26	26	7/9/2008
R. californica	UCOB_6200	7.40	0.19400	14.500	2325.000	11.3	73	9/28/2010
R. stoermeri	UCOB_6200	7.40	0.19400	14.500	2325.000	11.3	17	9/28/2010
R. lowei	UCOB_3065	7.98	0.05040	19.500	115.600	1.23	65	7/31/2008
R. californica	UCOB_3954	8.80	0.08210	53.900	167.000	5.06	46	7/29/2009
R. californica	UCOB_6345	7.20	0.01810	27.400	56.000	0.19	100	7/6/2010
R. californica	UCOB_3098	8.70	0.04850	41.900	218.000	1.04	93	9/23/2008
R. californica	UCOB_4294						20	9/21/2009
R. californica	UCOB_6204						61	9/15/2010
R. lowei	UCOB_3098	8.70	0.04850	41.900	218.000	1.04	93	9/23/2008
R. lowei	UCOB_4294						44	9/21/2009

R. lowei	UCOB_6204						61	9/15/2010
R. californica	UCOB_3081	7.79	0.06340	34.600	119.200	0.6	48	8/25/2008
R. lowei	UCOB_3081	7.79	0.06340	34.600	119.200	0.6	8	8/25/2008
R. californica	UCOB_7242	8.48	0.06810	37.800	85.000	0.69	59	7/12/2011
R. lowei	UCOB_7242	8.48	0.06810	37.800	85.000	0.69	59	7/12/2011
R. californica	UCOB_6212	6.88	0.02120	18.200	45.500	0.8	168	9/8/2010
R. californica	UCOB_7734	7.98	0.03460	26.200	145.000	2.96	99	9/21/2011
R. californica	UCOB_7788						115	10/12/2011
R. californica	UCOB_2997	7.80	0.07710	20.400	55.700	0.77	50	10/8/2008
R. californica	UCOB_6346	8.40	0.00900	16.000	130.000	2.12	193	8/31/2010
R. californica	UCOB_7722	7.85	0.02610	31.900	100.000	2.82	44	9/19/2011
R. lowei	UCOB_3001	8.53	0.04890	14.900	941.000	24	49	6/18/2008
R. californica	UCOB_3067						16	8/5/2008
R. californica	UCOB_3031	7.80	0.04040	33.800	115.100	0.66	18	6/17/2008
R. californica	UCOB_7718						15	9/13/2011
R. californica	UCOB_8469	7.69	0.01320	6.090	42.000	2.55	25	9/14/2011
R. californica	UCOB_6222	7.64	0.04340	72.700	584.000	95	32	8/17/2010
R. sp. 5	UCOB_6222	7.64	0.04340	72.700	584.000	95	177	8/17/2010
R. californica	UCOB_6223	7.40	0.01300	20.950	200.300	5.22	15	6/3/2010
R. californica	UCOB_3956	8.20	0.04740	22.900	75.700	7.37	46	8/4/2009
R. californica	UCOB_6354	7.80	0.06070	51.500	133.000	0.77	38	8/31/2010
R. californica	UCOB_7717	8.20	0.06040	27.000	65.800	7.93	67	9/7/2011
R. californica	UCOB_6355						25	9/1/2010
R. californica	UCOB_3057	8.07	0.02130	16.900	68.200	0.63	16	7/23/2008
R. californica	UCOB_7728	7.67	0.01880	17.400	49.000	0.67	15	10/12/2011
R. californica	UCOB_7741						154	10/4/2011
R. californica	UCOB_3066						25	8/4/2008
R. sp. 5	UCOB_3066						43	8/4/2008
R. californica	UCOB_6356	7.30	0.08320	12.700	98.000	2.56	7	6/9/2010
R. californica	UCOB_6357	7.30	0.08320	12.700	98.000	2.56	1	6/9/2010
R. californica	UCOB_3943	8.50	0.12600	38.850	141.000	154	78	5/26/2009
R. californica	UCOB_7126	8.40	0.02350	35.150	211.300	5.94	15	6/7/2011
R. californica	UCOB_3928						4	4/29/2009
R. californica	UCOB_7128	7.80	0.03640	36.600	45.000	0.36	16	6/7/2011
R. californica	UCOB_3010	8.50	0.05840	19.800	1417.000	214	1	5/14/2008
R. sp. 5	UCOB_3010	8.50	0.05840	19.800	1417.000	214	2	5/14/2008
R. californica	UCOB_3009	8.15	0.07540	22.200	1226.000	256	11	5/14/2008
R. sp. 5	UCOB_5796	7.63	0.22400	26.800	3193.000	1080	34	6/2/2010
R. californica	UCOB_7131	8.30	0.02820	52.400	428.000	37.7	25	5/19/2011
R. californica	UCOB_7132	8.08	0.01650	41.100	530.000	29.6	60	5/26/2011
R. sp. 5	UCOB_7133	7.99	0.12400	23.800	2763.000	806	32	5/17/2011
R. californica	UCOB_8748	7.20	0.03870	47.000	712.000	23.7	8	5/31/2012
R. sp. 5	UCOB_5801	7.30	0.01540	5.530	1028.000	244	32	5/27/2010
R. sp. 5	UCOB_5802	8.30	0.02420	24.800	1265.000	296	11	5/25/2010
R. sp. 5	UCOB_7135	8.40	0.01600	26.000	1213.000	243	8	5/17/2011
R. sp. 5	UCOB_2888						8	5/7/2008
R. californica	UCOB_3008	7.50	0.21600	18.800	1825.000	188	3	5/13/2008
R. sp. 5	UCOB_3008	7.50	0.21600	18.800	1825.000	188	15	5/13/2008
R. sp. 5	UCOB_3791	8.00	0.13300	26.300	2411.000	432	51	5/4/2009
R. californica	UCOB_5803	7.20	0.10800	39.100	2227.000	207	30	5/19/2010
R. sp. 5	UCOB_5803	7.20	0.10800	39.100	2227.000	207	10	5/19/2010

R. sp. 5	UCOB_5805	7.93	0.12600	15.300	1460.000	264	20	5/19/2010
R. californica	UCOB_7136	7.97	0.05960	40.700	316.000	51.3	40	5/25/2011
R. californica	UCOB_6359	7.30	0.01630	35.700	265.900	56.5	8	6/9/2010
R. californica	UCOB_6363						23	7/13/2010
R. californica	UCOB_6364						9	8/24/2010
R. californica	UCOB_7138	8.23	0.03735	36.400	130.000	51.2	22	5/18/2011
R. californica	UCOB_7139	8.14	0.03740	39.100	425.000	53.1	20	5/18/2011
R. californica	UCOB_6358	7.40	0.03750	41.300	2639.000	12.4	10	6/8/2010
R. californica	UCOB_7116	8.16	0.06450	45.750	279.000	13.1	19	6/1/2011
R. sp. 5	UCOB_2883						28	5/5/2008
R. sp. 5	UCOB_5813	7.80	0.04600	35.700	1394.000	86.5	66	5/26/2010
R. californica	UCOB_7113	8.01	0.03450	50.400	600.000	21.9	20	5/31/2011
R. californica	UCOB_7140	8.51	0.03050	40.500	551.000	57.3	37	5/24/2011
R. californica	UCOB_3809						22	5/5/2009
R. sp. 5	UCOB_3810	8.30	0.19300	11.200	1075.000	73.2	23	5/6/2009
R. californica	UCOB_7114	8.08	0.02530	46.000	355.400	3.43	27	5/31/2011
R. californica	UCOB_7125	8.31	0.02380	53.200	716.000	52.2	9	5/25/2011
R. sp. 5	UCOB_2465	8.10	0.00490	10.841	1248.333	246.45	13	6/4/2008
R. sp. 5	UCOB_2665						56	6/17/2007
R. californica	UCOB_2671	7.67	0.07460	18.396	1965.333	362.69	34	
R. californica	UCOB_2650	8.43	0.02870	9.100	512.667	103.93	45	
R. sp. 3	NRSA0562	8.67	0.01489	44.956	659.560	100.22	53	5/5/2009
R. sp. 4	NRSA1202	8.27	0.01171	7.655	768.000	155	79	10/24/2009
R. sp. 4	NRSA1205	8.25	0.01108	7.614	770.960	157.65	71	10/19/2009
R. sp. 2	NRSA1210	8.21	0.00988	8.220	772.930	159.92	32	
R. sp. 4	NRSA1210	8.21	0.00988	8.220	772.930	159.92	32	
R. sp. 4	NRSA1209	8.08	0.01413	7.976	685.020	160.21	34	10/12/2009
R. sp. 4	NRSA0676	8.37	0.03852	18.664	3847.590	72.11	52	6/29/2009
R. sp. 4	NRSA1214	8.08	0.01462	8.348	682.060	163.64	45	10/9/2009
R. sp. 4	NRSA1204	8.27	0.01546	7.767	773.920	156.61	50	10/25/2009
R. sp. 4	NRSA1203	8.29	0.01099	7.631	768.980	156.46	41	10/20/2009
R. sp. 3	NRSA0565	8.16	0.14977	39.708	1726.540	139.24	39	5/4/2009
R. californica	NRSA1181	8.44	0.02105	30.389	431.700	8.75	295	6/23/2009
R. lowei	NRSA1181	8.44	0.02105	30.389	431.700	8.75	295	6/23/2009
R. californica	NRSA0538	8.25	0.02135	14.533	370.680	33.41	90	7/24/2008
R. stoermeri	NRSA0538	8.25	0.02135	14.533	370.680	33.41	90	7/24/2008
R. californica	NRSA1183	8.21	0.02253	20.167	1359.560	442.48	53	5/27/2009
R. stoermeri	NRSA1183	8.21	0.02253	20.167	1359.560	442.48	53	5/27/2009
R. californica	NRSA0540	8.14	0.01358	17.827	206.750	3.43	179	9/23/2008
R. californica	NRSA1188	7.94	0.09594	31.415	95.000	0.5	82	8/10/2009
R. californica	NRSA1191	8.37	0.11384	28.366	281.610	8.53	101	7/7/2009
R. californica	NRSA1194	7.29	0.02791	13.846	68.040	3.02	88	8/18/2009
R. stoermeri	NRSA1197	8.40	0.00441	18.833	380.240	38.26	62	6/4/2009
R. californica	NRSA1198	8.16	0.02438	11.656	286.710	27.4	94	6/3/2009
R. sp. 3	NRSA0049	8.29	0.04554	13.099	1404.000	589.83	161	7/10/2008
R. sp. 3	NRSA0059	8.41	0.04151	3.171	661.200	112.53	115	6/18/2008
R. sp. 3	NRSA0047	8.43	0.08545	15.986	827.420	281.04	48	8/15/2008
R. sp. 1	NRSA0046	8.06	0.04433	8.802	423.440	92.87	55	8/12/2008
R. sp. 2	NRSA0922	8.26	0.03373	7.610	229.380	43.47	34	8/20/2009
R. sp. 3	NRSA0632	8.43	0.02931	8.336	485.030	65.56	60	6/30/2009
R. sp. 1	NRSA1084	8.00	0.03442	12.144	495.380	11.78	101	9/2/2009

R. sp. 1	NRSA1088	7.61	0.13649	7.018	187.130	8.48	61	9/30/2009
R. sp. 1	NRSA0476	8.09	0.08065	11.459	309.750	10.25	67	8/13/2008
R. sp. 1	NRSA0878	8.29	0.02860	5.972	326.070	7.03	36	8/12/2009
R. sp. 1	NRSA1091	7.69	0.13913	6.967	197.470	8.89	61	9/30/2009
R. sp. 1	NRSA0574	7.98	0.11174	10.399	322.680	19.7	137	6/3/2009
R. sp. 1	NRSA0415	7.68	0.10254	10.964	359.910	11.02	57	8/13/2008
R. sp. 1	NRSA1066	7.91	0.05274	11.599	319.300	11.07	72	8/17/2009
R. lowei	NRSA0849	8.23	0.04414	15.270	241.950	14.89	34	8/4/2009
R. sp. 2	NRSA0849	8.23	0.04414	15.270	241.950	14.89	34	8/4/2009
R. sp. 2	NRSA0027	8.17	0.05358	22.422	214.430	1.81	75	7/22/2008
R. lowei	NRSA0026	8.32	0.03684	48.049	135.500	5.11	46	7/2/2008
R. sp. 2	NRSA0026	8.32	0.03684	48.049	135.500	5.11	46	7/2/2008
R. lowei	NRSA0909	8.05	0.05943	17.794	275.790	17.38	35	9/2/2009
R. sp. 2	NRSA0909	8.05	0.05943	17.794	275.790	17.38	35	9/2/2009
R. sp. 1	NRSA0065	7.55	0.03076	6.300	197.000	22.69	201	6/25/2008
R. sp. 1	NRSA0068	8.47	0.04901	6.737	340.800	35.19	36	7/17/2008
R. sp. 3	NRSA0946	8.11	0.09281	6.365	385.660	19.33	45	6/10/2009
R. sp. 1	NRSA0453	7.81	0.01662	3.953	115.690	6.97	93	9/11/2008
R. sp. 1	NRSA0312	8.13	0.12113	5.741	650.980	198.93	55	9/23/2008
R. sp. 3	NRSA0312	8.13	0.12113	5.741	650.980	198.93	55	9/23/2008
R. sp. 3	NRSA0091	8.45	0.05268	21.040	852.640	190.34	70	8/25/2008
R. sp. 3	NRSA1235	8.08	0.14122	5.212	539.150	66.84	42	6/10/2009
R. sp. 3	NRSA1234	8.30	0.01929	6.486	546.740	123.23	54	9/16/2009
R. sp. 3	NRSA1113	8.93	0.03792	1.778	1297.430	268.34	90	9/16/2009
R. sp. 2	NRSA1226	8.30	0.31738	22.567	1259.640	240.56	31	7/13/2009
R. sp. 3	NRSA0796	8.32	0.08809	10.868	733.400	54.98	30	7/21/2009
R. sp. 2	NRSA0175	8.51	0.27863	3.426	1721.530	470.41	53	9/11/2008
R. sp. 3	NRSA0175	8.51	0.27863	3.426	1721.530	470.41	53	9/11/2008
R. sp. 2	NRSA0164	8.35	0.21589	18.792	1160.090	289.64	41	9/6/2008
R. sp. 2	NRSA0184	9.74	0.27577	3.414	2699.230	1173.21	35	7/31/2008
R. sp. 2	NRSA0167	8.48	0.04057	3.365	1713.000	504.57	103	7/17/2008
R. sp. 3	NRSA0167	8.48	0.04057	3.365	1713.000	504.57	103	7/17/2008
R. sp. 2	NRSA0179	8.41	0.08214	20.529	1240.740	388.83	50	9/15/2008
R. sp. 3	NRSA0179	8.41	0.08214	20.529	1240.740	388.83	50	9/15/2008
R. sp. 2	NRSA0158	8.52	0.11662	5.440	1478.000	389.99	65	6/25/2008
R. sp. 3	NRSA0158	8.52	0.11662	5.440	1478.000	389.99	65	6/25/2008
R. sp. 2	NRSA0147	8.79	0.03395	1.212	1738.000	516.93	88	7/8/2008
R. sp. 3	NRSA0147	8.79	0.03395	1.212	1738.000	516.93	88	7/8/2008
R. sp. 2	NRSA0178	8.46	0.17080	14.421	1284.590	339.41	72	9/17/2008
R. sp. 3	NRSA0178	8.46	0.17080	14.421	1284.590	339.41	72	9/17/2008
R. sp. 2	NRSA0965	8.54	0.15754	10.667	2129.440	545.48	165	6/18/2009
R. sp. 3	NRSA0965	8.54	0.15754	10.667	2129.440	545.48	165	6/18/2009
R. sp. 2	NRSA0964	8.43	0.19001	20.192	1288.070	248.37	55	6/25/2009
R. sp. 1	NRSA0020	8.11	0.01407	16.098	418.410	58.03	89	7/19/2008
R. sp. 1	NRSA0727	8.04	0.28995	11.509	364.790	24.65	209	7/7/2009
R. sp. 1	NRSA0729	7.95	0.18956	9.958	488.510	16.88	176	7/9/2009
R. sp. 1	NRSA0024	7.56	0.09058	10.623	349.300	50.94	97	7/27/2008
R. sp. 4	NRSA0687	7.96	0.16159	10.979	252.760	40.22	52	5/16/2009
R. sp. 4	NRSA0690	8.08	0.11684	17.227	227.420	31.17	30	5/21/2009
R. californica	NRSA0548	7.98	0.04838	34.677	167.620	9.58	51	5/24/2009
R. sp. 4	NRSA0686	8.15	0.01959	10.555	354.450	77.67	52	5/13/2009

R. sp. 2	NRSA0211	8.23	0.40951	31.249	303.720	37.08	53	8/5/2008
R. sp. 2	NRSA0223	8.43	0.01575	15.897	337.400	4.9	58	7/9/2008
R. sp. 2	NRSA0220	8.29	0.02347	40.905	264.400	7.95	32	8/7/2008
R. lowei	NRSA0225	8.60	0.13494	48.117	213.800	9.87	44	6/25/2008
R. sp. 2	NRSA0225	8.60	0.13494	48.117	213.800	9.87	44	6/25/2008
R. sp. 2	NRSA0244	8.26	0.10310	45.901	156.900	7.22	49	6/17/2008
R. californica	NRSA0207	8.09	0.19761	22.633	327.400	33.47	65	6/30/2008
R. sp. 5	NRSA0207	8.09	0.19761	22.633	327.400	33.47	65	6/30/2008
R. sp. 2	NRSA0252	7.71	0.12365	39.508	50.250	1.66	51	7/31/2008
R. sp. 5	NRSA0215	8.71	0.10050	32.532	1036.560	99.05	138	7/22/2008
R. californica	NRSA0230	8.07	0.04777	37.841	140.400	2.42	184	6/24/2008
R. californica	NRSA0251	7.59	0.02580	26.504	47.320	1.35	139	7/17/2008
R. sp. 1	NRSA0022	7.69	0.01958	6.535	50.810	5.96	41	7/18/2008
R. sp. 3	NRSA0019	8.09	0.04660	11.387	770.330	157.21	37	8/5/2008
R. sp. 1	NRSA0393	8.12	0.02821	2.264	796.030	83.12	44	9/23/2008
R. sp. 1	NRSA0427	8.04	0.03465	2.749	979.640	86.3	89	10/6/2008
R. sp. 3	NRSA0427	8.04	0.03465	2.749	979.640	86.3	89	10/6/2008
R. sp. 1	NRSA0426	7.73	0.01811	6.501	183.100	9.49	127	10/4/2008
R. lowei	NRSA0368	8.14	0.08884	43.200	203.100	12.83	93	7/21/2008
R. lowei	NRSA0349	7.45	0.02897	16.167	74.690	0.54	44	9/15/2008
R. sp. 2	NRSA0373	7.99	0.01490	18.938	69.980	1.34	31	7/9/2008
R. lowei	NRSA0359	7.89	0.07246	28.052	71.710	1.03	85	8/19/2008
R. californica	NRSA0845	7.67	0.06774	18.862	90.730	2.49	40	7/27/2009
R. lowei	NRSA0354	7.71	0.02750	16.105	73.850	0.97	131	9/11/2008
R. lowei	NRSA0357	8.00	0.05725	24.219	85.320	1.68	45	8/20/2008
R. lowei	NRSA0628	7.79	0.12212	38.898	83.790	2.35	158	7/6/2009
R. lowei	NRSA0367	7.68	0.03087	22.816	50.430	0.7	42	8/5/2008
R. sp. 2	NRSA0367	7.68	0.03087	22.816	50.430	0.7	42	8/5/2008
R. lowei	NRSA0365	8.02	0.02257	20.647	69.800	1.81	39	8/2/2008
R. lowei	NRSA1039	8.02	0.12443	19.406	388.920	42.8	92	9/11/2009
R. sp. 1	NRSA0779	8.04	0.07628	16.155	243.440	14.74	139	7/15/2009
R. sp. 1	NRSA0903	7.89	0.04937	2.952	203.280	35.03	89	8/25/2009
R. sp. 1	NRSA0904	8.06	0.06721	4.035	230.350	7.38	43	8/26/2009
R. sp. 1	NRSA1052	8.12	0.10431	4.133	580.320	111.66	30	9/18/2009
R. sp. 1	NRSA1042	7.76	0.09362	14.406	318.340	26.65	93	9/9/2009
R. sp. 1	NRSA0186	8.04	1.45554	3.014	824.220	37.4	95	8/5/2008
R. sp. 1	NRSA1237	7.94	0.10247	9.200	331.870	33.98	55	9/2/2009
R. sp. 1	NRSA0841	8.29	0.16023	4.318	552.670	55.83	71	8/10/2009
R. sp. 1	NRSA1050	7.72	0.04408	11.270	542.790	22.21	60	9/17/2009
R. sp. 1	NRSA1058	7.11	0.05564	5.453	52.780	6.18	379	9/26/2009
R. sp. 1	NRSA1059	6.67	0.02038	3.577	27.770	4.64	122	9/27/2009
R. sp. 4	NRSA0670						33	6/27/2009
R. sp. 1	NRSA0862	7.83	0.12768	6.415	490.440	19.93	57	8/23/2009
R. sp. 2	NRSA0271						36	9/21/2008
R. sp. 3	NRSA0825	8.14	0.40179	20.357	1877.330	786.25	39	7/22/2009
R. sp. 3	NRSA0987	8.14	0.06454	12.091	2143.450	1105.2	54	8/25/2009
R. sp. 2	NRSA0114	8.16	0.59224	53.217	635.630	26.71	55	7/30/2008
R. sp. 2	NRSA0302	8.39	0.03760	40.392	380.500	8.14	43	7/9/2008
R. sp. 3	NRSA0302	8.39	0.03760	40.392	380.500	8.14	43	7/9/2008
R. sp. 3	NRSA0293	8.58	0.07296	30.467	479.780	28.2	98	9/24/2008
R. sp. 1	NRSA0300	8.66	0.03342	35.874	522.100	36.21	35	7/10/2008

R. sp. 3	NRSA1162	8.17	0.06310	7.256	471.670	118.13	73	8/10/2009
R. sp. 3	NRSA1173	8.12	0.29298	11.958	1187.790	310.57	30	9/23/2009
R. sp. 1	NRSA1159	8.47	0.04975	7.158	487.470	125.53	47	8/11/2009
R. sp. 3	NRSA1159	8.47	0.04975	7.158	487.470	125.53	47	8/11/2009
R. sp. 3	NRSA1168	8.35	0.05586	7.210	488.460	124.36	61	8/13/2009
R. lowei	NRSA0306	8.28	0.07287	30.624	495.240	26.06	34	9/23/2008
R. sp. 1	NRSA0306	8.28	0.07287	30.624	495.240	26.06	34	9/23/2008
R. sp. 3	NRSA0306	8.28	0.07287	30.624	495.240	26.06	34	9/23/2008
R. sp. 1	NRSA0509	7.71	0.08241	15.676	108.810	1.49	288	9/2/2008
R. sp. 1	NRSA1206	7.94	0.01404	4.722	357.010	36.45	31	10/20/2009
R. lowei	NRSA1026	7.90	0.02577	7.209	155.120	11.42	34	9/27/2009
R. lowei	NRSA1025	7.98	0.09232	15.836	290.300	29.26	54	9/25/2009
R. sp. 2	NRSA1025	7.98	0.09232	15.836	290.300	29.26	54	9/25/2009
R. lowei	NRSA1149	7.50	0.05066	20.293	87.860	1.43	90	10/11/2009
R. lowei	NRSA1148	7.52	0.05364	19.687	89.250	2.5	80	10/6/2009
R. sp. 1	NRSA1006	7.69	0.01441	4.545	115.110	9.51	57	9/14/2009
R. sp. 1	NRSA0659	7.83	0.05518	7.729	335.410	21.89	59	7/14/2009
R. sp. 2	NRSA0576	7.89	0.14523	22.302	136.120	1.8	48	6/8/2009
R. sp. 3	NRSA0576	7.89	0.14523	22.302	136.120	1.8	48	6/8/2009
R. sp. 3	NRSA0757	8.69	0.05538	7.052	503.340	111.09	98	7/18/2009
R. sp. 3	NRSA0655	8.31	0.16805	10.167	409.850	42.9	32	7/15/2009
R. sp. 2	NRSA0751	8.62	0.04040	8.669	453.940	92.64	48	7/20/2009
R. sp. 3	NRSA0751	8.62	0.04040	8.669	453.940	92.64	48	7/20/2009
R. sp. 2	NRSA0575	7.45	0.04850	14.243	53.930	1.99	59	6/9/2009
R. sp. 3	NRSA0334	8.39	0.01331	0.934	1655.000	869.03	100	7/15/2008
R. sp. 3	NRSA0591	8.31	0.09545	4.267	572.570	141.54	32	6/21/2009
R. sp. 3	NRSA0755	8.59	0.08768	5.420	550.800	129.39	61	7/19/2009
R. sp. 1	GSN00871						88	9/1/1999
R. sp. 1	GS017363	5.80	0.03000	10.000	144.600	14	276	10/12/1994
R. sp. 1	GS017254	5.82	0.03000	8.900	351.000	32	38	9/13/1994
R. sp. 1	GS017443	7.53	0.08000	12.000	364.000	41	77	9/8/1995
R. sp. 1	GS017273	6.24	0.04000	16.000	233.000	14	300	9/14/1994
R. sp. 1	GS017283	6.66	0.01000	10.000	254.000	16	121	9/15/1994
R. sp. 1	GS017163						163	9/27/1994
R. sp. 1	GS017173						140	9/28/1994
R. sp. 1	GS017203	6.88	0.01000	9.500	211.000	7	124	9/6/1994
R. sp. 1	GS017193	8.34	0.01000	6.100	318.000	17	81	10/13/1994
R. sp. 1	GS100570						57	8/27/2002
R. sp. 1	GS017213	6.41	0.01000	6.200	308.000	13	58	9/7/1994
R. lowei	GS001361						30	9/13/1994
R. sp. 2	GS167613						390	7/24/2007
R. sp. 2	GS181541						256	8/4/2008
R. sp. 1	GS008363	8.03	0.01000	3.800	522.300	100	67	8/12/1993
R. sp. 1	GS008763	8.05	0.02000	7.000	1338.000	580	203	6/21/1995
R. sp. 1	GS008712	8.28	0.03000	3.600	1093.000	410	129	6/20/1995
R. sp. 1	GS008732	7.59	0.01000	3.200	1006.000	340	66	6/20/1995
R. sp. 1	GS008752	7.89	0.03000	1.400	845.000	260	67	6/21/1995
R. sp. 1	GS008843	7.98	0.02000	6.900	719.000	30	144	7/10/1995
R. sp. 1	GS008900	7.48	0.07000	2.200	323.000	24	64	7/24/1995
R. sp. 1	GS008273	7.68	0.03000	6.300	967.000	71	176	8/19/1993
R. sp. 1	GS008003	8.03	0.04000	5.800	301.000	19	44	7/19/1993

R. sp. 3	GS008003	8.03	0.04000	5.800	301.000	19	44	7/19/1993
R. sp. 1	GS008530	8.17	0.01000	2.600	60.200	5.9	69	7/14/1994
R. sp. 1	GS008880	7.75	0.08000	5.000	279.000	21	120	7/18/1995
R. sp. 1	GS008503	8.04	0.05000	10.000	653.000	27	262	7/11/1994
R. sp. 1	GS008283	8.59	0.01000	5.200	323.000	15	47	8/19/1993
R. sp. 3	GS008283	8.59	0.01000	5.200	323.000	15	47	8/19/1993
R. sp. 1	GS008193	7.80	0.01000	4.400	511.000	12	71	8/11/1993
R. sp. 1	GS008513	7.63	0.05000	5.700	292.000	13	261	7/12/1994
R. sp. 1	GS028173	7.30	0.04400	11.504	593.000	17.823	71	9/23/1996
R. sp. 1	GS028183	7.90	0.02000	10.000	551.000	18	89	7/3/1996
R. sp. 1	GS028213						92	10/2/1996
R. sp. 1	GS028253	8.08	0.01000	4.875	243.000	13.666	78	9/11/1996
R. sp. 1	GS028593	7.10	0.01000	5.444	259.000	31.801	91	7/20/1998
R. sp. 1	GS028273	7.60	0.01000	11.390	267.000	9.904	150	9/13/1996
R. sp. 1	NJP024	7.90	0.02400	11.975	273.000	17.511	78	9/30/2009
R. sp. 1	GS028303	8.50	0.06000	7.600	228.000	19	287	7/11/1996
R. sp. 1	GS028313	7.41	0.07000	12.055	224.000	22.841	146	9/10/1996
R. sp. 1	GS117832						152	7/29/2003
R. sp. 1	GS194093						182	7/29/2009
R. lowei	GSN99372						163	9/19/2002
R. sp. 1	GS028353	7.90	0.01400	7.355	657.000	27.569	30	10/1/1996
R. sp. 1	GS028363						31	9/27/1996
R. sp. 1	GS028373						176	9/26/1996
R. sp. 1	GSN00487						39	9/14/1999
R. sp. 1	GSN00487						39	9/14/1999
R. sp. 1	GS028383						106	10/1/1996
R. sp. 1	GSN24572	7.60	0.05500	11.036	290.000	23.97	417	8/22/2000
R. sp. 1	GSN24564	7.80	0.03700	16.161	319.000	23.11	90	8/22/2000
R. sp. 1	GSN97323						67	7/16/2002
R. sp. 3	GSN97323						67	7/16/2002
R. sp. 1	GSN24423	8.10	0.02300	12.613	510.000	29.16	187	8/31/2000
R. sp. 1	GSN24415	8.10	0.02000	12.435	159.000	24.55	252	8/31/2000
R. sp. 1	GS137426						178	8/12/2004
R. sp. 1	GSN24568	8.30	0.04400	10.340	305.000	25.15	219	8/29/2000
R. sp. 1	GSN24524	8.42	0.03300	12.847	539.000	25.57	131	8/17/2000
R. sp. 1	GSN24528	7.80	0.04800	11.631	480.000	37.8	140	8/17/2000
R. sp. 1	GSN24588	7.80	0.02900	15.441	190.000	11.5	101	8/16/2000
R. sp. 1	GSN24520	8.10	0.13300	15.071	269.000	18.53	354	8/16/2000
R. sp. 1	GSN24411	8.00	0.17700	15.266	329.000	23.67	409	8/23/2000
R. sp. 1	GSN24596	7.70	0.08100	15.640	248.000	17.95	481	8/24/2000
R. sp. 1	GSN24435	7.90	0.02500	10.483	321.000	21.98	339	8/15/2000
R. sp. 1	GS011053	7.00	0.02000	4.500	74.000	11	213	7/9/1993
R. sp. 1	GS011013	8.60	0.49000	3.300	535.000	22	50	7/6/1993
R. sp. 1	GS011163	8.30	0.01000	7.600	401.000	23	120	8/2/1993
R. sp. 1	GS011043	7.70	0.01000	8.000	640.000	30	103	7/8/1993
R. sp. 1	GS011263	8.10	0.02000	12.000	592.000	26	125	5/31/1994
R. sp. 1	GS011453	7.00	0.07000	4.900	157.000	17	91	8/16/1994

R. sp. 1	GS011563	8.00	0.16000	5.100	832.000	52	62	6/12/1995
R. sp. 1	GS018083	7.30	0.04000	4.100	613.000	180	147	8/25/1993
R. sp. 3	GS018083	7.30	0.04000	4.100	613.000	180	147	8/25/1993
R. sp. 1	GS018943	7.40	0.01000	8.300	130.000	11	179	8/9/1995
R. sp. 1	GS018883	7.20	0.13000	8.300	250.000	15	238	6/20/1995
R. sp. 1	GS018123	7.80	0.01000	11.000	590.000	31	40	9/8/1993
R. sp. 1	GS018253	8.10	0.07000	3.700	518.000	43	52	9/9/1993
R. sp. 1	GS018273	8.10	0.03000	8.700	612.000	15	49	9/10/1993
R. sp. 1	GS018474	8.00	0.01000	3.900	350.000	16	61	6/23/1994
R. sp. 1	GS018913	7.00	0.02000	11.000	144.000	12	54	6/23/1995
R. sp. 1	GS018643	7.90	0.41000	12.000	420.000	31	127	8/30/1994
R. sp. 1	GS018613	8.30	0.07000	7.700	247.000	23	107	8/29/1994
R. sp. 1	GS018603	8.10	0.29000	11.000	399.000	26	181	8/29/1994
R. sp. 1	GS018893	7.31	0.15000	8.400	236.000	20	70	6/20/1995
R. sp. 1	GS018503	7.60	0.03000	7.600	211.000	8	46	8/16/1994
R. sp. 1	GS018653	7.50	0.28000	10.000	496.000	20	154	8/31/1994
R. sp. 1	GS018023	7.90	0.16000	5.600	280.000	18	44	7/29/1993
R. sp. 1	GS018723	7.30	0.01000	13.000	330.000	16	37	8/16/1994
R. sp. 1	GS018823	7.60	0.03000	9.800	318.000	15	131	8/30/1994
R. sp. 1	GS018763	7.30	0.02000	13.000	359.000	14	121	8/25/1994
R. sp. 1	GS108598						35	5/14/2003
R. sp. 1	GS108612						53	5/19/2003
R. sp. 1	GS108456						52	5/15/2003
R. sp. 1	GS108588						94	5/15/2003
R. sp. 1	GS108498						44	5/13/2003
R. sp. 1	GS024143	7.00	0.15100	14.292	170.000	18.6	41	6/17/1997
R. sp. 1	GSN00879	8.10	0.28200	5.892	432.000	51.189	136	6/22/1999
R. sp. 1	GSN17517	7.90	8.64800	11.924	1710.000	277.96	31	6/13/2000
R. sp. 1	GSN00900	8.10	0.20600	8.296	316.000	7.622	104	6/17/1999
R. sp. 1	GSN17587	7.60	0.00700	4.929	310.000	9.13	46	6/8/2000
R. sp. 1	GS026273	8.00	0.03100	1.908	325.000	16.117	35	7/22/1998
R. sp. 3	GS026273	8.00	0.03100	1.908	325.000	16.117	35	7/22/1998
R. sp. 1	GS026121	7.90	0.01000	7.300	775.000	250	86	7/10/1996
R. sp. 1	GSL00758	8.32	0.04200	2.386	304.000	10.327	56	5/13/1997
R. sp. 1	GSL00764	8.11	0.05000	2.370	270.000	26.271	47	6/10/1997
R. sp. 1	GS141653	8.04	0.47900	7.884	866.000	50.554	139	8/10/2004
R. sp. 3	GSN00287						37	7/14/1999
R. sp. 1	GSL0W193	7.77	0.02000	7.500	669.000	38	65	6/23/1994
R. sp. 3	GSL0W193	7.77	0.02000	7.500	669.000	38	65	6/23/1994
R. sp. 3	GSN95843						36	7/23/2002
R. sp. 3	GSN95859						119	7/23/2002
R. sp. 1	GSL0W155	7.56	0.06000	15.000	519.000	50	52	6/14/1993
R. sp. 1	GSL0W166	7.68	0.10000	11.000	512.000	26	76	6/29/1993
R. sp. 1	GSL0W197	8.30	0.03000	2.600	551.000	55	181	6/15/1994
R. sp. 3	GSL0W197	8.30	0.03000	2.600	551.000	55	181	6/15/1994
R. californica	GS133935						85	7/12/2004

R. sp. 1	GSL00096	8.13	0.01000	4.000	262.000	10	46	5/22/1996
R. sp. 1	GSL00098	8.02	0.07000	3.900	294.000	19	141	6/17/1996
R. sp. 3	GSL00108	8.46	0.04000	1.700	306.000	28	57	6/18/1996
R. sp. 1	GSL00110	8.31	0.01000	2.500	328.000	33	80	6/20/1996
R. sp. 1	GS186515						107	8/13/2008
R. sp. 3	GSN62437						106	7/17/2000
R. sp. 1	GS181825						55	8/19/2008
R. sp. 1	GS004573	8.40	0.10000	3.700	644.000	51	80	5/22/1995
R. sp. 3	GS004573	8.40	0.10000	3.700	644.000	51	80	5/22/1995
R. sp. 1	GS118850						107	9/9/2003
R. sp. 1	GS138571						93	9/1/2004
R. sp. 1	GS138390						120	8/30/2004
R. sp. 1	GS138308						169	8/28/2004
R. sp. 1	GS138276						83	8/28/2004
R. sp. 1	GS138427						81	8/31/2004
R. sp. 1	GS004543	8.22	0.13000	2.300	828.000	74	74	5/17/1995
R. sp. 1	GS004173	8.71	0.01000	5.400	501.000	17	99	6/5/1993
R. sp. 1	GS138224						91	8/26/2004
R. sp. 1	GS138241						93	8/26/2004
R. sp. 1	GS138719						33	8/26/2004
R. sp. 1	GS004054	8.09	0.06000	5.900	656.000	29	48	5/23/1993
R. sp. 3	GS108896						154	6/11/2003
R. sp. 1	GSN98782						81	9/9/2002
R. sp. 3	GSN98782						81	9/9/2002
R. sp. 1	WRD0009						92	9/18/2007
R. sp. 3	WRD0009						92	9/18/2007
R. sp. 1	GS138095						70	8/24/2004
R. sp. 1	GS138065						126	8/23/2004
R. sp. 1	WRD0013						88	9/19/2008
R. sp. 3	WRD0013						88	9/19/2008
R. sp. 1	GS138605						97	9/1/2004
R. sp. 1	GS138618						113	9/1/2004
R. sp. 1	GS138646						108	8/24/2004
R. sp. 3	GSL00606	7.87	0.04400	4.034	401.000	21.443	51	9/26/1997
R. sp. 1	GSX007390	7.96	0.01180	10.456	539.000	27.79	30	8/18/2010
R. sp. 1	GS139365	7.90	0.17220	10.500	542.000	52.38	58	8/24/2004
R. sp. 3	GS019543	8.38	0.04000	12.000	1137.000	280	61	6/22/1995
R. sp. 1	GS019133						81	7/16/1993
R. sp. 3	GS019033	8.08	0.22000	1392.000	907.000	180	55	6/24/1993
R. sp. 3	GS019533						119	6/21/1995
R. sp. 1	GS019523	8.09	0.05000	7.200	748.000	110	416	6/21/1995
R. sp. 1	GS019173	8.04	0.06000	11.000	366.000	12	155	7/21/1993
R. sp. 1	GSL00112	8.13	0.01000	8.900	315.000	9.4	38	9/23/1996
R. sp. 1	GSL00369	8.10	0.03200	21.506	607.000	23.776	146	9/24/1997
R. sp. 1	GSL00370	7.70	0.04200	13.094	438.000	9.326	68	9/16/1997
R. sp. 1	GS168150	7.70	0.11500	18.440	521.000	10.425	55	8/10/2007

R. sp. 1	GSL00372	7.90	0.03000	20.937	519.000	37.053	102	8/16/1997
R. sp. 1	GSL00373	7.90	0.01000	18.689	689.000	72.523	112	8/16/1997
R. sp. 1	GSL00119	8.30	0.03000	11.000	345.000	13	71	9/16/1996
R. sp. 1	GS136769						82	8/17/2004
R. sp. 1	GSL00344						45	8/12/1997
R. sp. 3	GS168316						65	7/31/2007
R. sp. 1	GSL00350						38	8/13/1997
R. sp. 1	GSL00352						55	8/20/1997
R. sp. 1	GSL00127	8.22	0.12000	33.000	650.000	34	44	9/13/1996
R. sp. 1	GSL00130	7.84	0.08000	18.000	634.000	100	156	9/5/1996
R. sp. 3	GSL00201	7.79	0.02000	2.500	592.000	17	48	5/14/1996
R. sp. 1	GSL00386	7.40	0.01400	4.030	498.000	7.886	81	9/23/1997
R. sp. 1	GSL00139	8.10	0.16000	17.000	654.000	75	35	9/19/1996
R. sp. 1	GSL00151	8.56	0.10000	9.300	436.000	34	35	9/24/1996
R. sp. 1	GS194198						156	8/6/2009
R. sp. 3	GSL00400	7.81	0.15600	6.985	579.000	26.702	60	8/28/1997
R. sp. 1	GSL00403	8.05	0.05800	16.109	680.000	39.98	60	8/26/1997
R. sp. 1	GSL00005	8.09	0.05000	17.000	688.000	53	99	9/5/1996
R. sp. 1	GSL00405	8.21	0.04800	11.299	593.000	50.837	31	8/11/1997
R. sp. 1	GSL00407	7.71	0.01000	12.578	685.000	43.215	31	8/27/1997
R. sp. 1	GSL00412	8.05	0.04000	10.652	639.000	26.962	38	8/19/1997
R. sp. 1	GSL00015	8.33	0.04000	11.000	540.000	38	60	8/7/1996
R. sp. 1	GSL00432	8.15	0.09300	13.725	603.000	38.263	95	7/22/1997
R. sp. 3	GSL00432	8.15	0.09300	13.725	603.000	38.263	95	7/22/1997
R. sp. 1	GSN21680	8.13	0.21800	13.349	830.000	25.51	54	7/18/2000
R. sp. 1	GSN20566						108	7/14/2000
R. sp. 1	GSN20615						154	7/14/2000
R. sp. 1	GSN20746	7.81	1.67300	6.236	962.000	76.45	97	7/13/2000
R. sp. 1	GSN20651	7.92	0.05700	6.476	700.000	29.89	38	7/11/2000
R. sp. 1	GSN20961	7.52	0.09400	6.335	778.000	37.68	214	7/17/2000
R. sp. 1	GSN20540	7.80	0.09200	6.638	780.000	42.45	98	7/17/2000
R. sp. 1	GSN20321	7.90	0.21400	11.010	650.000	70.77	75	7/12/2000
R. sp. 1	GSN20643	7.62	0.05900	4.032	760.000	154.08	56	7/12/2000
R. sp. 1	GSN20691	7.86	0.03800	5.522	548.000	44.58	118	7/13/2000
R. sp. 1	GSN20626	7.70	0.18900	9.229	735.000	57.99	106	7/13/2000
R. sp. 1	GSN21095	7.81	7.80000	0.182	11.114	595	73	7/11/2000
R. sp. 1	GSN21654	7.60	0.14400	10.160	644.000	53.4	81	7/13/2000
R. sp. 1	GSN21085	7.95	0.94600	6.325	1010.000	90.04	64	7/14/2000
R. sp. 1	GSN20418	7.83	0.02800	5.683	755.000	66.29	76	7/21/2000
R. sp. 1	GS138143						75	8/25/2004
R. sp. 1	GSN21099	7.41	0.05700	3.035	647.000	17.68	76	7/19/2000
R. sp. 1	GSN21233	7.90	0.04400	9.425	694.000	38.11	71	7/20/2000
R. sp. 1	GSN20318	8.10	0.04900	8.591	728.000	41.4	95	7/10/2000
R. sp. 1	GSN21624	7.83	0.00700	6.877	794.000	71.4	159	7/21/2000
R. sp. 1	GSN20679	7.90	0.05500	6.999	798.000	63.47	74	7/24/2000
R. sp. 1	GSN20683	7.90	7.92000	0.037	5.982	729	50	7/25/2000

R. sp. 1	GSL00037	8.01	0.06000	8.400	752.000	56	98	9/12/1996
R. sp. 1	GSL00308	7.40	0.12200	11.991	654.000	32.778	103	8/8/1997
R. sp. 1	GSL00044						53	9/10/1996
R. sp. 1	GSL00320	7.80	0.01000	6.342	560.000	42.766	50	8/5/1997
R. sp. 1	GSL00340	7.90	0.04200	8.068	616.000	32.673	36	8/8/1997
R. lowei	GSN59205	8.28	0.01200	18.362	196.000	19.64	74	8/23/2000
R. sp. 2	GSN59205	8.28	0.01200	18.362	196.000	19.64	74	8/23/2000
R. sp. 3	GSN59205	8.28	0.01200	18.362	196.000	19.64	74	8/23/2000
R. lowei	GSN58747						35	8/22/2000
R. sp. 3	GSN58747						35	8/22/2000
R. lowei	GSN58753	8.83	0.00900	8.879	339.000	54.5	41	8/21/2000
R. sp. 3	GSN58753	8.83	0.00900	8.879	339.000	54.5	41	8/21/2000
R. sp. 3	GSN58755						136	8/24/2000
R. sp. 3	GSN58761	8.89	0.00500	4.399	636.000	170.86	74	8/25/2000
R. sp. 3	GSN58765						35	8/25/2000
R. sp. 3	GS007313	8.80	0.20000	14.000	1040.000	190	215	7/12/1994
R. sp. 3	GSN94057	8.40	0.22800	8.737	443.000	66.747	298	9/9/1998
R. sp. 3	GS007173						117	9/16/1993
R. sp. 3	GS007223						155	9/22/1993
R. sp. 3	GS135378						133	8/9/2004
R. sp. 3	GS007041	8.50	0.05000	29.000	1070.000	310	55	7/28/1993
R. sp. 1	GS198786						144	9/2/2010
R. sp. 1	GS021123	8.02	0.01000	8.800	374.000	6.5	81	9/21/1994
R. sp. 1	GS021154	8.05	0.27000	8.600	378.000	5.5	77	9/7/1994
R. sp. 1	GS021281	8.09	0.02000	12.000	369.000	4.916	39	9/13/1994
R. sp. 1	GS171574						37	8/28/2007
R. sp. 1	GS002003	7.40	0.02000	13.000	511.000	16	51	6/14/1993
R. sp. 1	GS002351	7.20	1.30000	8.200	624.000	70	40	8/17/1994
R. sp. 3	GS002371	7.80	0.11000	6.000	271.000	31	38	8/19/1994
R. sp. 1	GS010131	8.40	0.08000	30.000	131.000	3.6	30	7/19/1994
R. sp. 2	GS010093	8.70	0.03000	23.000	337.000	50	65	9/7/1993
R. sp. 4	GS010093	8.70	0.03000	23.000	337.000	50	65	9/7/1993
R. sp. 4	GS010063	8.40	0.02000	13.000	289.000	56	61	9/9/1993
R. sp. 2	GS010353	7.50	0.02000	58.000	112.000	2.2	51	7/11/1995
R. sp. 4	GS010423	8.10	0.33000	23.000	428.000	61	67	8/28/1995
R. sp. 3	GS025131	8.30	0.03000	21.000	1280.000	460	213	8/9/1996
R. sp. 3	GS114046	8.50	0.01000	18.000	589.000	54	54	8/18/2003
R. californica	GS145584						64	10/26/2004
R. sp. 4	GSL00802						52	1/30/1996
R. sp. 3	GS111770						134	7/31/2003
R. sp. 3	GSN80914	8.20	0.01740	9.294	618.000	25.89	151	7/11/2001
R. sp. 2	GSN24747	8.20	0.07600	7.759	394.000	10.51	32	8/17/2000
R. sp. 2	GSN24106	7.90	0.07700	13.410	1300.000	161.15	224	8/28/2000
R. sp. 3	GSN24106	7.90	0.07700	13.410	1300.000	161.15	224	8/28/2000
R. sp. 3	GS111847						157	7/29/2003
R. lowei	GS000001	8.40	0.23000	20.000	139.000	14	30	9/1/1993

R. sp. 2	GS000001	8.40	0.23000	20.000	139.000	14	30	9/1/1993
R. lowei	GS000323						58	7/21/1994
R. sp. 2	GS000323						58	7/21/1994
R. californica	GS000503						30	9/13/1995
R. lowei	GS000313						213	7/20/1994
R. sp. 2	GS000313						213	7/20/1994
R. sp. 3	GS000293	8.40	0.12000	24.000	800.000	190	112	7/15/1994
R. lowei	GS000283						82	7/14/1994
R. sp. 2	GS000283						82	7/14/1994
R. californica	GS000133	8.10	0.03000	28.000	560.000	130	65	9/13/1993
R. sp. 5	GS000463	8.80	0.01000	22.000	211.000	6.5	31	10/2/1995
R. sp. 2	GS000433						298	9/22/1995
R. sp. 2	GS000453	7.20	0.04000	12.000	217.000	16	66	9/25/1995
R. californica	GS000243						124	7/6/1994
R. sp. 3	GS000243						124	7/6/1994
R. lowei	GS000633	8.40	0.01000	17.000	668.000	67	197	10/8/1996
R. sp. 2	GS000633	8.40	0.01000	17.000	668.000	67	197	10/8/1996
R. sp. 5	GS000633	8.40	0.01000	17.000	668.000	67	197	10/8/1996
R. sp. 3	GS000573						99	10/13/1995
R. lowei	GS000403	8.30	0.02000	19.000	849.000	150	56	10/6/1995
R. sp. 2	GS000403	8.30	0.02000	19.000	849.000	150	56	10/6/1995
R. sp. 5	GS133940						46	7/12/2004
R. californica	GS029013	7.90	0.01000	22.000	95.060	1.9	116	8/21/1996
R. lowei	GS029013	7.90	0.01000	22.000	95.060	1.9	116	8/21/1996
R. lowei	GS029033	7.97	0.01000	33.000	110.000	1.2	120	8/9/1996
R. californica	GS029073	7.97	0.01000	34.000	161.000	2.9	158	8/5/1996
R. californica	GS137234						225	8/22/2004
R. sp. 2	GS030193	7.70	0.01000	13.702	48.000	0.698	149	8/31/1998
R. sp. 2	GS030043						147	8/21/1997
R. sp. 2	GS030203	8.00	0.04200	19.373	156.000	6.153	137	8/26/1998
R. sp. 2	GS030213	7.80	0.01000	17.233	140.000	8.683	87	8/24/1998
R. sp. 2	GS030393	7.90	0.02000	29.000	228.000	17	277	8/19/1996
R. lowei	GS030173	7.70	0.01000	13.373	263.000	36.524	34	8/4/1997
R. lowei	GS120732						185	9/4/2003
R. sp. 2	GS120479						58	8/28/2003
R. lowei	GS001153						106	9/2/1993
R. sp. 2	GS120119						56	8/15/2003
R. sp. 2	GSN63853						57	11/3/2000
R. lowei	GS119512						60	8/20/2003
R. lowei	GS119791						60	8/26/2003
R. lowei	GSN63842						31	9/27/2000
R. sp. 2	GSN63842						31	9/27/2000
R. lowei	GSN63884						31	9/28/2000
R. lowei	GS151671						126	10/6/2004
R. lowei	GS009453	8.00	0.01000	36.000	368.000	46	44	9/7/1994
R. sp. 2	GS009453	8.00	0.01000	36.000	368.000	46	44	9/7/1994
R. lowei	GS009103	8.19	0.02000	7.300	496.000	36	30	8/10/1993
R. sp. 2	GS009213						62	9/15/1993

R. lowei	GS009051	8.19	0.01000	16.000	404.000	37	30	7/22/1993
R. lowei	GS167625						31	7/16/2007
R. lowei	GS009043	8.44	0.07000	26.000	553.000	57	47	7/19/1993
R. lowei	GS009013						45	7/8/1993
R. sp. 2	GS009013						45	7/8/1993
R. sp. 2	GS009423	8.37	0.01000	9.000	204.000	15	81	8/29/1994
R. sp. 2	GS009151	8.31	0.05000	21.000	440.000	40	39	8/26/1993
R. lowei	GS009023	8.44	0.05000	28.000	490.000	48	255	7/13/1993
R. sp. 2	GS009023	8.44	0.05000	28.000	490.000	48	255	7/13/1993
R. lowei	GS001081						39	8/25/1993
R. sp. 3	GS001013						264	8/18/1993
R. sp. 3	GS001401	8.90	1.90000	19.000	634.000	28	109	8/8/1995
R. lowei	GS016023	7.33	0.26000	40.000	394.000	14	208	8/10/1993
R. lowei	GS016403	7.65	0.01000	18.000	72.200	1.2	296	8/11/1995
R. sp. 2	GS016403	7.65	0.01000	18.000	72.200	1.2	296	8/11/1995
R. lowei	GS145387						233	9/28/2004
R. sp. 2	GS145387						233	9/28/2004
R. lowei	GS180413						219	8/15/2007
R. lowei	GS016063	7.44	0.04000	21.000	230.000	17	105	8/18/1993
R. californica	GS029263						91	9/12/1997
R. sp. 5	GS029263						91	9/12/1997
R. sp. 1	GSL0W173	7.76	0.13000	7.800	457.000	32	35	7/22/1993
R. sp. 3	GSL0W177	8.02	0.04000	1.700	522.000	33	43	8/4/1993
R. sp. 1	GSN82509	8.04	0.02030	3.507	602.000	57.47	43	7/24/2001
R. sp. 3	GSN82509	8.04	0.02030	3.507	602.000	57.47	43	7/24/2001
R. sp. 3	GSN00323						64	7/13/1999
R. sp. 3	GSL0W179	7.96	0.09000	7.800	448.000	29	72	7/20/1993
R. sp. 3	GS007193						109	9/17/1993
R. sp. 1	GS110931						74	6/23/2003
R. sp. 3	GS110931						74	6/23/2003
R. sp. 1	GS111138						181	7/1/2003
R. sp. 3	GS111138						181	7/1/2003
R. sp. 1	GSN82595	8.17	0.01220	7.750	849.000	32.95	102	7/10/2001
R. sp. 3	GSN82595	8.17	0.01220	7.750	849.000	32.95	102	7/10/2001
R. sp. 1	GS141632						68	8/10/2004
R. sp. 1	GSN82569	7.93	0.01980	4.013	653.000	32.24	91	7/10/2001
R. sp. 3	GSN82569	7.93	0.01980	4.013	653.000	32.24	91	7/10/2001
R. sp. 3	GSN55367	8.27	0.01700	6.768	712.000	34.87	99	7/11/2000
R. sp. 1	GSN82599	7.91	0.22200	4.695	970.000	54.57	44	7/17/2001
R. sp. 1	GSN82620	8.12	0.01740	9.138	754.000	58.14	43	7/13/2001
R. sp. 1	GSN82700	8.11	0.03680	8.946	724.000	42.86	80	7/16/2001
R. sp. 1	GSN82823	8.12	0.02590	9.503	776.000	41.05	33	7/11/2001
R. sp. 3	GSN82813	8.03	0.07970	4.071	685.000	40.1	62	7/17/2001
R. sp. 1	GS141456						31	8/12/2004
R. sp. 3	GS141456						31	8/12/2004
R. californica	GS029313						93	9/3/1997
R. lowei	GS029313						93	9/3/1997
R. sp. 1	GSN00335						33	7/7/1999
R. sp. 3	GSN00335						33	7/7/1999
R. sp. 1	GS111025						99	6/27/2003
R. sp. 3	GS111025						99	6/27/2003

R. sp. 1	GSN82489	8.03	0.06150	5.997	675.000	35.16	83	7/19/2001
R. sp. 3	GSN82489	8.03	0.06150	5.997	675.000	35.16	83	7/19/2001
R. sp. 1	GSN82658	7.77	0.02420	6.039	600.000	73.44	113	7/18/2001
R. sp. 1	GS110992						270	7/3/2003
R. sp. 3	GS110992						270	7/3/2003
R. sp. 3	GSN24735	7.90	0.01600	10.024	486.000	42.98	56	8/1/2000
R. lowei	GSN23649	8.30	0.01000	2.905	453.000	42.07	65	8/1/2000
R. sp. 3	GSN23649	8.30	0.01000	2.905	453.000	42.07	65	8/1/2000
R. lowei	GSN24707	8.30	0.03500	9.933	399.000	43.48	144	8/2/2000
R. sp. 3	GSN24707	8.30	0.03500	9.933	399.000	43.48	144	8/2/2000
R. sp. 2	GSN24737	8.60	0.02200	8.565	322.000	40.17	130	8/2/2000
R. sp. 3	GSN24737	8.60	0.02200	8.565	322.000	40.17	130	8/2/2000
R. sp. 3	GSN24733	8.20	0.01800	8.784	334.000	39.67	51	8/3/2000
R. sp. 1	GS011183						169	8/5/1993
R. sp. 3	GS007161						79	9/15/1993
R. sp. 1	GS011103						102	7/21/1993
R. sp. 3	GS111104						115	7/8/2003
R. sp. 3	GS116019						190	7/8/2003
R. sp. 1	GS111000						103	7/7/2003
R. sp. 3	GS111000						103	7/7/2003
R. sp. 1	GS011093						219	7/20/1993
R. lowei	GSN23866	8.10	0.01700	14.457	1090.000	135.64	229	7/21/2000
R. sp. 3	GSN23866	8.10	0.01700	14.457	1090.000	135.64	229	7/21/2000
R. lowei	GSN23870	7.80	0.02100	12.553	1010.000	109.2	91	7/24/2000
R. sp. 3	GSN23870	7.80	0.02100	12.553	1010.000	109.2	91	7/24/2000
R. sp. 3	GSN24081	8.40	0.01500	8.772	540.000	113.14	127	7/25/2000
R. sp. 3	GSN23886						188	8/14/2000
R. sp. 1	GSN24717	8.29	0.02500	12.019	336.000	9.4	49	8/15/2000
R. sp. 3	GSN24749						46	8/22/2000
R. sp. 3	GSN24181	8.90	0.26200	8.066	1250.000	111.06	108	8/14/2000
R. sp. 1	GS011213						198	8/12/1993
R. sp. 3	GS007253						119	9/27/1993
R. sp. 1	GSN23874	7.70	0.04400	10.653	399.000	19.97	163	7/31/2000
R. lowei	GSN23814	7.90	0.06000	16.795	916.000	33.95	73	7/26/2000
R. sp. 3	GSN23814	7.90	0.06000	16.795	916.000	33.95	73	7/26/2000
R. lowei	GSN23819	8.00	0.09000	12.378	578.000	25.33	81	7/20/2000
R. sp. 3	GSN23819	8.00	0.09000	12.378	578.000	25.33	81	7/20/2000
R. sp. 3	GSN24731	8.10	0.08600	11.003	658.000	33.26	37	7/19/2000
R. sp. 3	GSN23906	8.30	0.08300	14.074	814.000	39.18	127	7/26/2000
R. sp. 1	GS111098						127	7/9/2003
R. lowei	GSN23800	8.00	0.00800	6.292	347.000	9.5	268	8/7/2000
R. sp. 3	GSN23800	8.00	0.00800	6.292	347.000	9.5	268	8/7/2000
R. lowei	GSN24745	8.40	0.00600	9.195	418.000	14.08	218	8/9/2000
R. sp. 2	GSN24745	8.40	0.00600	9.195	418.000	14.08	218	8/9/2000
R. sp. 3	GSN24745	8.40	0.00600	9.195	418.000	14.08	218	8/9/2000
R. lowei	GSN24741	8.30	0.00700	6.755	406.000	11.69	217	8/9/2000
R. sp. 2	GSN24741	8.30	0.00700	6.755	406.000	11.69	217	8/9/2000
R. sp. 3	GSN24741	8.30	0.00700	6.755	406.000	11.69	217	8/9/2000
R. sp. 1	GS112206						49	7/23/2003
R. sp. 1	GS112384						118	7/31/2003
R. sp. 1	GS112673						41	7/28/2003

R. lowei	GS016133						157	7/27/1994
R. lowei	GS016081						36	7/25/1994
R. lowei	GS016143						444	7/27/1994
R. sp. 2	GS016143						444	7/27/1994
R. lowei	GS016153						351	7/27/1994
R. lowei	GS145513						256	9/27/2004
R. sp. 2	GS145513						256	9/27/2004
R. lowei	GS145405						139	9/27/2004
R. sp. 2	GS145405						139	9/27/2004
R. lowei	GS016163						96	7/27/1994
R. lowei	GS016253						108	8/1/1994
R. lowei	GS016263						88	8/1/1994
R. lowei	GS145479						170	9/14/2004
R. sp. 2	GS145479						170	9/14/2004
R. lowei	GS016273						284	8/8/1994
R. sp. 2	GS016273						284	8/8/1994
R. lowei	GS145561						75	9/17/2004
R. lowei	GS145527						134	9/24/2004
R. lowei	GSN63846						82	9/29/2000
R. sp. 2	GSN63846						82	9/29/2000
R. lowei	GS119810						62	8/21/2003
R. lowei	GSN63834						109	9/25/2000
R. lowei	GS122292						91	9/24/2003
R. sp. 2	GSN63900						38	9/26/2000
R. sp. 3	GS001111						78	8/31/1993
R. lowei	GS001073						32	8/25/1993
R. sp. 2	GS001073						32	8/25/1993
R. sp. 2	GS001333	8.00	0.02000	20.000	71.000	0.7	162	9/1/1994
R. sp. 1	GS019273						83	7/18/1994
R. californica	UCOB_2483	7.46	0.01570	7.022	2976.667	1138.67	63	7/4/2007
R. californica	UCOB_2485	6.65	0.13560	13.200	2966.667	1137.66	155	6/29/2007
R. sp. 5	UCOB_2683	7.87	0.08920	8.903	3143.333	1016.81	78	6/13/2008
R. sp. 5	UCOB_2685	8.31	0.03110	8.201	1075.667	245.88	92	6/18/2008
R. californica	UCOB_2837	7.46	0.01880	4.550	346.000	10.19	23	11/2/2008
R. californica	UCOB_2710	8.07	0.01660	8.973	381.667	23.53	13	6/12/2008
R. sp. 5	UCOB_2710	8.07	0.01660	8.973	381.667	23.53	63	6/12/2008
R. sp. 5	UCOB_2714	8.63	0.02640	6.404	910.000	244.13	16	6/16/2008
R. stoermeri	UCOB_2527	7.69	0.00108	11.459	410.667	58.43	54	6/25/2007
R. californica	UCOB_2732	7.97	0.00340	12.498	714.667	197.96	244	6/3/2008
R. sp. 5	UCOB_2552	7.89	0.01510	0.154	3406.667	1015.29	20	6/12/2007
R. californica	UCOB_2557	7.91	0.00650	14.492	680.667	146.16	85	6/15/2007
R. sp. 5	UCOB_2557	7.91	0.00650	14.492	680.667	146.16	67	6/15/2007
R. californica	UCOB_2559	7.98	0.03270	0.814	1318.667	244.35	23	6/11/2007
R. sp. 5	UCOB_2559	7.98	0.03270	0.814	1318.667	244.35	5	6/11/2007
R. californica	UCOB_2638	7.31	0.00710	4.942	453.000	18.64	84	11/7/2007
R. sp. 5	UCOB_2760	7.75	0.16990	6.713	2720.000	352.54	55	6/17/2008
R. sp. 5	UCOB_2589	8.31	0.00410	7.246	872.667	254.41	17	7/3/2007