Documentation and Identification of the One Known Freshwater Sponge Discovered in the California Delta

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Abstract: Extensive sampling for freshwater sponges was carried out at a representative site in the California Delta system, USA. The results provide the first documented account of freshwater sponges in this habitat. Using scanning electron microscopy *Heteromeyenia tubisperma* Potts, 1881 was identified, and the results presented aim to contribute to the identification of gemmules of this sponge. This study describes the first known occurrence of a single sponge species at this particular habitat.

Keywords: Biodiversity, freshwater sponge, gemmules, California Delta, *Heteromeyenia tubisperma*.

INTRODUCTION

Freshwater sponges are an important component of aquatic ecosystem filtering particles of a smaller size range than other benthic invertebrates. The study of freshwater sponges has become increasingly popular as more is known about their ability to filter large volumes of water together with their importance as bioindicators in aquatic environments [1, 2]. In spite of this, there have been relatively few sponge studies in western parts of the North American Continent with most freshwater sponge data collected from habitats in the eastern, southern, and mid-western United States [3-9]. Initial observations were made in the early part of the twentieth century [10-12], but these observations do not include detailed gemmule information, which is now recognized as one of the most important criteria in describing freshwater sponges. Following this no rigorous sampling of freshwater sponges in California has been published, so their contribution to the biodiversity and function in freshwater systems remains unclear. The states of California, Washington and Oregon contain many freshwater sites where sponges might be expected to occur. A very small number of fossil sponges have been reported [13], but there are relatively few records for the whole of the states west of the Rockies. Given the scarcity of data on overall species distribution patterns in Californian waters, the present study examines the sponges found in the California Delta as a representative sampling site within this area.

The California Delta consists of miles of waterways providing theoretically suitable substrates for sponges including aquatic macrophytes and permanent solid material. In similar ecosystems, freshwater sponges may exhibit localised abundance [2] with two species, *Ephydatia fluviatilis* Linnaeus and *Spongilla lacustris* Linnaeus well-distributed in the northern hemisphere. Previously reported sampling in the California Delta has failed to record sponge species; this is likely because freshwater sponges are often overlooked due to their small size and crust-like colonies [14]. In this investigation, extensive sampling, specifically for sponges was conducted in the California Delta followed by an ultrastructure study of individuals to unambiguously resolve their taxonomic status.

Taxonomic confusion can lead to the incorrect identification of sponge specimens and lead to an under-estimation of the contribution of sponges to an aquatic environment [15]. Although sponges are the least complicated of multicellular animal taxa, confusion arises as shape, color, and size of freshwater sponges are variable within a single species [15] making identification from gross morphology unreliable. More accurate identification is derived from studying the degree of ultrastructure complexity found in sponge skeletal elements. Thus, in this study photomicrographs from Scanning Electron Microscope (SEM) are used to provide increased magnification, and an accurate method used for measurement and identification.

Components of the sponge skeleton provide the basis of a classical method of identification. Siliceous spicules bound together with collagen are the two components of a freshwater sponge skeleton [16, 17]; megascleres, microscleres and gemmoscleres are the three types of spicules. Examining only the larger megascleres from a sponge skeleton with light microscopy does not necessarily provide distinguishing characteristics. Instead, the morphological characters on which freshwater sponge classification relies most heavily are associated with gemmules and include gemmoscleres and the gemmule foramen [16, 18, 19]. Using increased magnification, these can be analyzed as diagnostic traits at the genus and species level. Thus in this work, we have used SEM techniques to measure and characterize gemmule structure for identification purposes.

MATERIALS AND METHODOLOGY

A study site was chosen in this representative area of California (Fig. **1**) in a 1.5 ha expanse of open water, called

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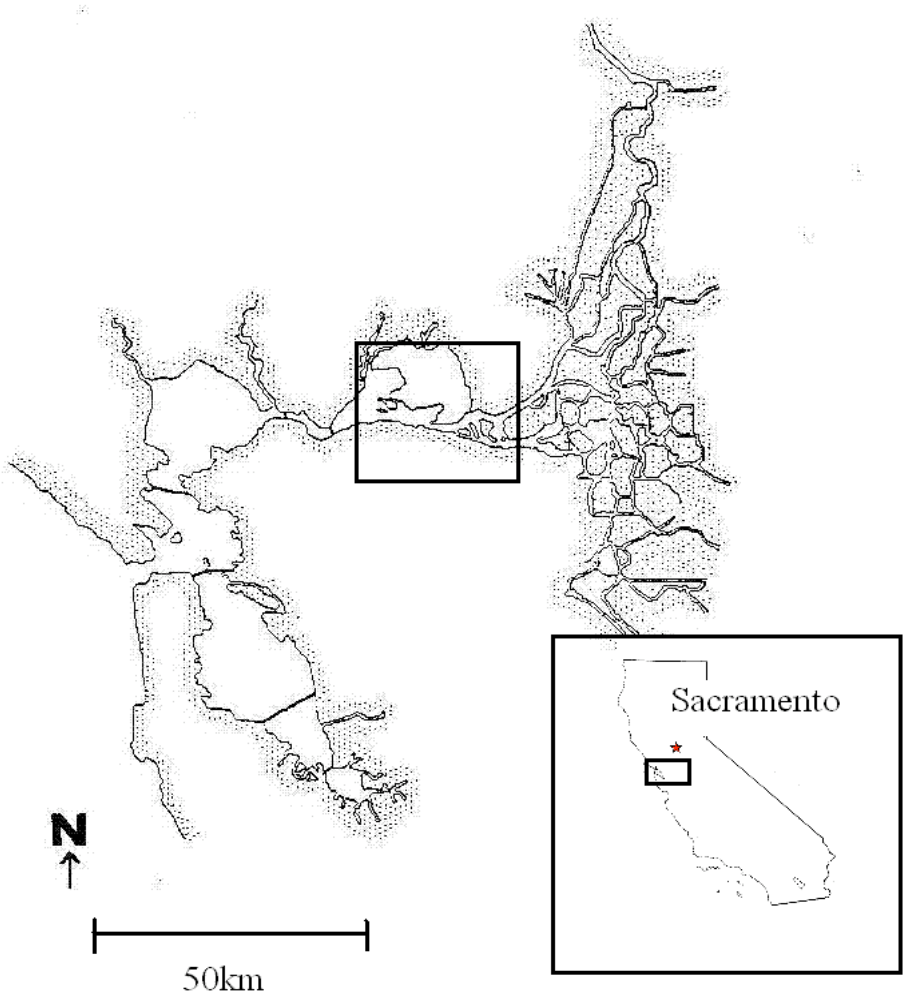

Fig. (1). Map of California (inset) with location of Delta area. California Delta showing route of major waterways and areas of sampling.

the "Big Break", south of Jersey Island, which is approximately 50 km upstream from San Francisco Bay, together with the adjacent Big Break Marina, 38.01N, -121.73W. Both open water and marina sites were sampled once or twice a month for eighteen months for a total of 24 months. Water temperature and salinity were measured using a mercury thermometer and refractometer each month.

An underwater video camera was lowered to locate sponge specimens *in situ*. In open water, aquatic macrophytes and submerged pieces of wood were collected using a net and examined for sponges and/or gemmules. Locations of large specimens or areas of high sponge abundance were noted. Patches of sponge were also measured and collected from Big Break Marina where they grew on submerged floats up to the surface of the water. To prevent sponge samples from becoming mixed, smaller isolated sponges were collected from the floats manually (i.e. without using nets or other auxiliary devices). Individual samples were obtained by cutting a small section of the polystyrene float with the sponge attached. Sponges were preserved in 70% ethyl alcohol and returned to the laboratory for identification following standard keys [20].

Sponges were identified using growth form, consistency, skeletal megascleres and microscleres, gemmule architecture and gemmosclere morphology and compared to published descriptions [21]. Observations were made with both light microscope and scanning electron microscopy (SEM). For SEM, sponge fragments were fixed in 0.25% glutaraldehyde in filtered Delta water and cleaned gemmules postfixed in 1% osmium tetroxide. These were dehydrated in ethanol alcohol, and sponge fragments and entire gemmules were sputter coated with gold/palladium.

RESULTS

The Delta sampling areas (Fig. **1**) were between 1 ppt salinity and <0.3 ppt upstream. Water temperatures ranged between 10 ^{o}C and 35 ^{o}C. Sponges were patchily distributed within the sampling areas, in summer quadrats included an average of 4 sponges in 0.25 m^2. Sponges formed thin encrustations up to about 400 mm in diameter with a thickness of approximately 5 mm.

Examples of the spicule morphology of the preparations analyzed are presented in Fig. (**2**). Following examination,

all spicules were consistent with the sponge species *Heteromeyenia tubisperma* Potts. Its characteristic traits include the large irregular encrusting growth form [18] and a color range of whitish to grey. The sponge skeleton displayed an irregular network of scattered spicules (Fig. **2A**) and had a fragile consistency.

Megascleres were amphioxea, slender and sharply pointed (Fig. **2A**). They were straight to slightly bent and width measurements ranged from 8-10 µm consistent with previous records [18]. However, megasclere length was longer than 200 µm suggested by other authors [18]. There were fewer microscleres, which were slender and covered with curved spines (Fig. **2B**). Spines on microscleres were curved in one direction which is consistent with previous descriptions [22]. There were too few intact microscleres to determine length but widths were 4-5 µm. Numerous spherical gemmules were found ranging in size from 410 µm to 550 µm in diameter (Fig. **2C**). Gemmules had a well developed pneumatic layer packed with gemmoscleres.

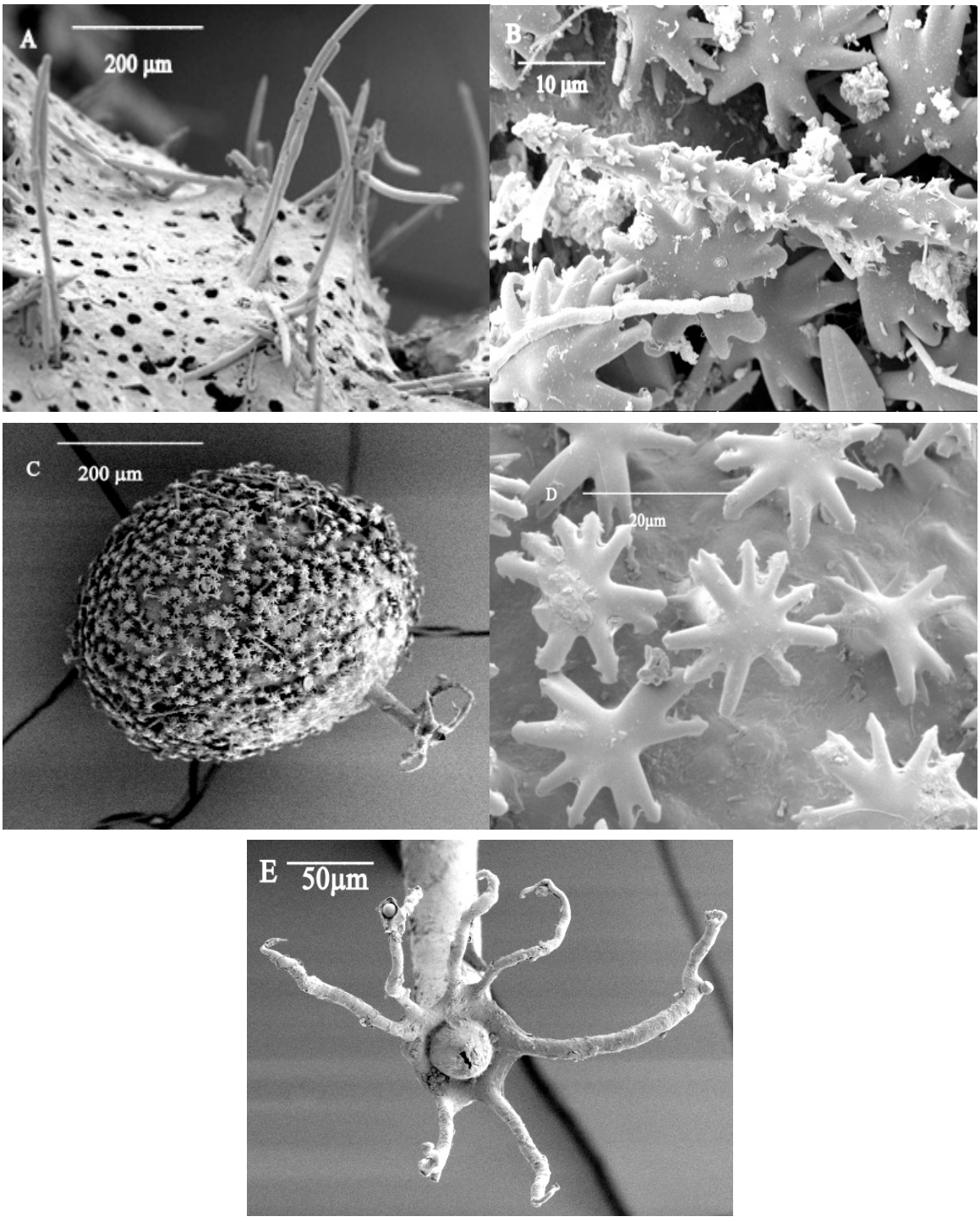

Fig. (2). Ultrastructure of *Heteromeyenia tubisperma.* (**A**) Megascleres and spongin arrangement of sponge. (**B**) Microsclere. (**C**) Gemmule. (**D**) Enlarged rotules of gemmoscleres. (**E**) Enlarged foraminal tubule of gemmule.

Individual gemmoscleres were birotulate with shafts that were cylindrical and stout. Rotules were of equal diameter with 7-13 numerous deeply incised and recurved teeth (Fig. **2D**). Microspines were seen at the ends of the rotule teeth (Fig. **2D**). This species is characterized by a long, slender foraminal tubule and cirrous appendages from the gemmule (Fig. **2E**). These features can be seen clearly in Fig. (**2E**). The foraminal tubule ranged from 57 μm to 170 μm long. The number of cirrous appendages varied, between four and seven. The cirrous projections were tubular rather than irregular.

DISCUSSION AND CONCLUSION

Initially we used using light microscopy to analyze spicules and identify all sponges specimens collected. Although the resolution of a light microscope is sometimes sufficient for the morphological analysis of sponge spicules, scanning electron microscopy permitted the three-dimensional specific arrangement of gemmoscleres and shape of both the foraminal tubule and cirrous appendages to be characterized. Foraminal tubule and cirrous appendage details are pivotal as only the *Heteromeyenia* genus of freshwater sponges have the distinctive tubule characteristics. There is a minor difference in the number of cylindrical cirrous appendages compared to other published records [18, 22]. Also, the tubule length is less than 0.5 times the gemmule diameter, a figure suggested by other workers [14, 18]. However, it has been reported that size of gemmule features may change with season [18]. Despite these differences, gemmular structure is clearly that of *Heteromeyenia tubisperma*. In a related species with a tubule, *H. tentasperma,* the cirrous projections are irregular rather than cylindrical [16]. Other species widely distributed in temperate regions such as *Spongilla lacustris* Linnaeus and sponges of the genus *Ephydatia* produce gemmules without a tubule and appendages [18].

The SEM techniques provided an accurate method for the measurement of the sponge features and confirmed the presence of the sponge species *H. tubisperma* in this ecosystem. The characteristic features that were described may aid future investigators in identifying this species.

The rigorous sampling methods and SEM analysis conducted in this study identified only one species of freshwater sponge was present in this habitat, although we believe that the diverse Delta substrates sampled have the potential to support other sponge species. However, following the extensive temporal and spatial data collection, it seems likely that if other species were present in this habitat they would have been identified by the methods outlined here. The sole occurrence of *H. tubisperma* may indicate this species outcompetes other freshwater sponge species for substrate in this habitat. Although it is unusual to find only one species of freshwater sponge in such an ecosystem, other workers have found similar results [1]. A closely related freshwater sponge species, *Corvoheteromeyenia heterosclera* Ezcurra de Drago was the dominant species found in shallow coastal ponds in South America [1] demonstrating the occurrence of another species specific environment.

In this study we report the first scientific account with clear images of a freshwater sponge in the California Delta, which supports initial observations [10-12]. Included in these initial accounts are brief observations of freshwater sponges in the region, however, no gemmule ultrastructure data was included. Previously, no sponges have been reported in the California Delta. The genus *Heteromeyenia* has previously been reported in eastern parts of North American Continent [18, 22] and *H. tubisperma* reported in Canada [23]. This species is reported to be one of the four most common species in the USA [20]. This study extends the range of *H. tubisperma* from eastern North America to California. As the Delta site was chosen as a representative area of California then it is possible that other freshwater ecosystems also have the potential of supporting sponges. Without knowing the full extent of the current distributions of native freshwater sponges, it is not possible to identify changes in distribution over time. Further extensive studies are recommended to target other representative areas in California.

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