

## Article

# Aquatic Insects in Habitat-Forming Sponges: The Case of the Lower Mekong and Conservation Perspectives in a Global Context

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**Abstract:** Shallow water sponges settled on a raft along the Pong River (Lower Mekong Basin, Thailand) were investigated to highlight the taxonomic richness, composition, relative abundance and lifestyle of sponge-dwelling aquatic Insecta. The three-dimensional biogenic structures of the model sponges hosted 4 orders of Insecta, belonging to 10 families and 19 genera/species, able to strictly coexist at the level of the sponges in aquiferous canals and/or at the body surface, and/or dwelling in the extracellular matrix. On the basis of the identified 379 larvae and pupae, Trichoptera and Diptera were found to be the dominant inhabitants of *Corvospongilla siamensis* (Demospongiae: Spongillida), endemic to Southeast Asia. In the focused lotic ecosystem, dominated by soft bottoms, sponges play a functional role. Insecta use sponges as a substratum, nursery ground, food source, and shelter microhabitat, protecting them from predation and environmental aggression. Moreover, their feeding behavior indicates the insects' adaptive traits to recycle sponge siliceous spicules as a source of exogenous material to strengthen the larval–pupal cases and the digestive system. The results of the Thai sponge model contribute to the inventory of global engineering species richness, ecosystem types, and biogeographic diversity, thus raising awareness for freshwater biodiversity conservation. In this regard, the present data, along with the worldwide inventory, focus on sponges as (a) key habitat-forming species for aquatic insect assemblages, (b) ecosystem engineers in river/lake/wetland ecosystems, providing water purification, the processing of organic matter, recycling of nutrients, and freshwater–terrestrial coupling, and (c) promising candidates in restoration projects of tropical freshwater ecosystems by bioremediation.

**Keywords:** entomofauna diversity; tropical Insecta–Porifera association; global inventory; larval microhabitat and behavior; restoration and bioremediation



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## 1. Introduction

Spongillida (Demospongiae) inhabit and settle on a wide array of natural and artificial substrata in lotic and lentic water and wetlands in all continents, except Antarctica [1–3]. In the food web of freshwater ecosystems, sponges play a functional role as filter feeders, consuming the pelagic resources linking the pelagic and benthic trophic webs [4]. A sponge can filter up to  $35 \text{ mL min}^{-1}(\text{cm sponge})^{-3}$  [5]. This water volume is related to the sponge volume, oscule diameter, and water pumping rates, and could be affected by the temperature, food concentration, suspended sediment concentration, water flow, and viscosity [6–8]. This behavior was highlighted also in stressed conditions of sponge microcosms living in water rich with organic and bacterial loads in polluted sites during in situ experimental sponge aquaculture [9].

During their long-lasting lifecycle, continental sponges' growth forms range from encrusting to massive and arborescent in the sessile adult phase, with a body size from a few millimeters to more than 1 m in diameter [1,2,10].

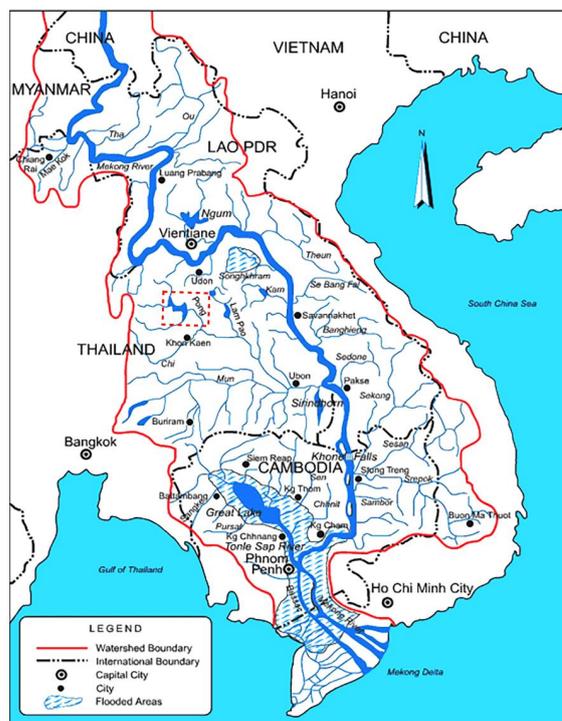
It is well known that the Spongillida (families Lubomirskiidae, Metaniidae, Potamolepidae, and Spongillidae) are associated with a diversified entomofauna worldwide, i.e., Ephemeroptera, Plecoptera, Odonata, Hemiptera, Megaloptera, Neuroptera, Trichoptera, Lepidoptera, Coleoptera, and Diptera, plus several other phyla ranging from bacteria to fishes and amphibians [1,2,11–13]. However, little is known about the diversity of sponge-dwelling aquatic insects and their relationship in Southeast Asia [14].

Investigations were carried out in the Lower Mekong Basin (Thailand, Oriental–Himalayan Region) to study (a) the composition, relative abundance, and taxonomic richness of sponge-dwelling aquatic insects, (b) their behavioral and ecological relationship, and (c) their biogeographic diversity. The data of insect–sponge associations are discussed also in comparison with the global dataset to highlight the sponges’ ecosystem services and to raise awareness of the biodiversity conservation of these peculiar living freshwater habitats.

## 2. Materials and Methods

### 2.1. Study Area

This study was carried out in the Pong River ( $16^{\circ}46'19.9''$  N,  $102^{\circ}38'3.25''$  E) which is a tributary of the Lower Mekong Basin (Figure 1) by the largest hydrographic basin of Northeastern Thailand. This regulated river provides an important water resource for agriculture, domestic uses, aquaculture, recreation, electricity generation, and industrial purposes, especially in Khon Kaen Province. Water is released from the outlet of the Ubolratana Dam to the lower part of the Pong River for water management, such as supplying water for agriculture and preventing water degradation by the operation of the Electricity Generating Authority Thailand, both in the wet (June–November) and dry (December–May) seasons. This eutrophic river, dominated by silty bottoms and having the most altered water quality of this region, since 1993, has been polluted from multiple sources, e.g., agricultural run-off, a pulp and paper mill, and a fertilizer factory [15,16]. Nevertheless, the water quality was in a generally fair condition, according to the surface water quality standard and water quality index [17].



**Figure 1.** Map of the Lower Mekong River hydrographic basin with the study site along Pong River (indicated by a red dashed-line square) in Northeastern Thailand, Southeast Asia (modified from van Zalinge et al. [18]).

The study site, Ban Huai Sai, is located at 165 m asl ( $16^{\circ}46'19.9''$  N,  $102^{\circ}38'3.25''$  E) ~1.5 km downstream from the outlet of the Ubolratana Dam (Figure 2). The riverbed is characterized by a maximum depth of 8 m and width of 70 m. The submerged substrata range from silty bottoms, timbers, and aquatic plants to artificial manufacture along the banks, e.g., fish cage nets, submerged structures of rafts, and nylon lines. The riparian vegetation mainly consists of the rain tree, *Samanea saman* (Jacq.) Merr. The aquatic vegetation mainly consists of *Colocasia* sp., *Hydrilla verticillata* (L. f.) Royle, *Cyperus* sp., and *Nephrolepis* sp. Sponges of the order Spongillida (Demospongiae) were found on the twigs and the bottom of the riverbed [19]. Flourishing and abundant sponge populations were also settled on the submerged artificial substrata, floating raft construction, buoys, and recreational fishing nylon lines trapped in the raft buoys at the study site (Figure 2a).



**Figure 2.** View of the study site. (a) *Corvospongilla siamensis* on artificial substrata. (b) Schematic diagram of a raft, submerged structures, and nylon lines colonized by sponges (orange) at 0.3–1 m water depth in the study site.

## 2.2. Sampling Methods

Fourteen specimens of sponges were collected in February 2008 (dry season, northeast monsoon) at two water depths, i.e., 0.3 m from the submerged raft construction (seven sponges), and 1 m on the nylon lines (seven sponges) (Figure 2b). These specimens were ascribed to *Corvospongilla siamensis*, Manconi & Ruengsawang, 2012. The identification of the sponges was based on the growth form, skeletal architecture, and microtraits. Detailed analyses of the gemmular theca and its gemmuloscleres, together with the skeletal microscleres and megascleres, were carried out [20–22]. All the sponges considered in the study were covered with a hand net of 450  $\mu$ m mesh size, carefully detached from the substrate, and then placed into plastic bags. To allow most of the aquatic insects to leave their hosts, each sample with its river water was placed in a plastic tray for half an hour (ambient temperature), and then it was preserved in 70% ethanol. Each sponge

was photographed in the field. In the laboratory, the samples were washed through a 500 µm mesh-size sieve, and the associated insects remaining on the surface of the sponges and sieve were collected by forceps. Each sponge was also carefully dissected to collect the insects that remained within its body. All the aquatic insects were sorted, identified to the lowest possible taxonomic level, and counted by screening at the microscope. The taxonomic identification of the insects from Ban Huai Sai was based on Morse et al. [23], Epler [24], Sangpradub and Boonsoong [25], and Cranston [26]. The similarities in the aquatic insect composition between the screened sponge specimens were analyzed by cluster analysis, using Jaccard's similarity index, by PC-ORD software version 5.10.

The gut contents of the dominant insect taxa were investigated qualitatively by light microscopy for their primary dietary components. Thirty specimens of each taxon were dissected under a stereomicroscope; their digestive tracts were mounted on slides and examined under a compound microscope. The cases of the trichopterans ( $n = 15$ ) and tubes of the chironomids ( $n = 15$ ) were examined and photographed by a scanning electron microscope (SEM, LEO model 1450VP) to determine whether these insects use the sponge spicules to construct their cases or tubes.

The water quality parameters were evaluated at two shallow water depths, (0.3 m and 1 m), during the dry season–northeast monsoon. The water quality parameters, such as dissolved oxygen (DO), electrical conductivity, total dissolved solids (TDS), water temperature, pH and flow velocity, were measured in the field, along with the collections of sponges at each depth, by portable digital instruments (a dissolved oxygen meter YSI model 57 (Yellow Spring Instrument Co. Inc., Yellow Spring, MP, USA); conductivity meter Fisher Scientific model 09-326-2 (Fisher Scientific International Inc., Pittsburgh, PA, USA); pH meter HACH sension<sup>TM</sup>1 (HACH Company, Loveland, CO, USA); Genuine Gurley<sup>®</sup> current meter model D625 digital pygmy meter with Model 1100 Flow Velocity Indicator (Gurley Precision Instruments, New York, NY, USA)). The suspended solids (SS), turbidity, nitrate, and phosphate were measured in the laboratory by the HACH DR/2010 Spectrophotometer (HACH Company, Loveland, CO, USA). The biological oxygen demand (5 days, 20 °C) (BOD<sub>5</sub>) was also evaluated. The difference in the environmental parameters between the two water depths was determined by the Mann–Whitney U test with SPSS for Windows software, version 16.

### 3. Results

#### 3.1. Physicochemical Parameters

The mean values of most of the physicochemical parameters were not significantly different between 0.3 m and 1 m, except flow velocity and DO, which were significantly higher at 1.0 m ( $p < 0.05$ , Supplementary Materials Table S1). Due to unavailable data from the deep zone, the comparative analyses on the suspended solids, turbidity, nitrate, and phosphate at the different water depths were not carried out.

#### 3.2. Composition and Abundance of Associated Aquatic Insects

A total of 379 larvae and pupae of aquatic insects belonging to 4 orders, 10 families, and 19 taxa were recorded, by microscopical screening, from 14 sponge samples at two water depths at Ban Huai Sai (Table 1; Figures 3–6). The dominant larvae colonizing *C. siamensis* were Trichoptera (48.81%) and Diptera (47.75%) (Figure 3b–d), whereas Neuroptera and Ephemeroptera were less represented. The relative abundance of associated insect taxa varied from 3 to 94 individuals per sponge specimen and ranged from 140 specimens (14 taxa) at 0.3 m depth to 239 specimens (16 taxa) at 1 m depth. The egg jelly envelopes of *Povilla heardi* Hubbard, 1984 (Ephemeroptera, Polymitarcyidae) were also found on the sponge surface (Figure 6a,b).

The most dominant taxa in the Pong River were *Ecnomus* spp., followed by *Amphipsyche meridiana* Ulmer, 1909 (Trichoptera), *Polypedilum* sp., and *Xenochironomus* sp. (Diptera), making up 77.8% of the specimens. The larval stages of first three genera are clingers, but *Xenochironomus* is a burrower in sponges. Eleven taxa were shared by both water depths, i.e.,

*Polypedilum* sp. was present in all the samples, followed by *Ecnomus* and *Xenochironomus*, respectively. The chironomid larvae *Ablabesmyia* and *Dicrotendipes* were found from six out of the seven sponges at the 0.3 m water depth. The larvae of *Sisyra* sp. were only 2.3% ( $n = 9$ ), the pupae of chironomids 2.3% ( $n = 9$ ), and the caddisflies 1% ( $n = 4$ ) were also recorded (Table 1). The cluster analysis, based on Jaccard’s similarity index, relying on presence/absence data, showed differences in the aquatic insect composition among the fourteen sponge samples and separated them into two main clusters (Figure 7). One cluster comprised the shallow-water samples (0.3 m) and the other comprised all but one of those from the deep water (1 m).

**Table 1.** Checklist of aquatic insects associated with *Corvospongilla siamensis* in the Pong River (Lower Mekong, Thailand). Total number of larvae and/or pupae in each sponge sample at two water depths. Presence of eggs is indicated by asterisk (\*).

Taxa	Number of Individuals														Total
	Depth 0.3 m							Depth 1.0 m							
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	
<b>Ephemeroptera</b>															
Baetidae gen. sp.	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
Caenidae															
<i>Caenodes</i> sp.	-	2	-	-	-	1	-	-	-	-	-	-	-	-	3
Polymitarcyidae															
<i>Povilla heardi</i>	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-
<b>Neuroptera</b>															
Sisyridae															
<i>Sisyra</i> sp.	-	3	1	-	1	-	-	2	-	1	1	-	-	-	9
<b>Trichoptera</b>															
Ecnomidae															
<i>Ecnomus</i> spp.	-	8	3	1	4	1	4	21	27	12	7	1	2	1	92
Hydropsychidae															
<i>Amphipsyche meridiana</i>	-	-	-	-	-	-	-	62	20	-	-	-	-	2	84
Hydroptilidae gen. sp.	-	1	-	-	-	-	-	4	1	1	-	-	-	-	7
Trichoptera fam. gen.	-	-	-	-	-	-	1	-	-	1	-	-	-	-	2
<b>Diptera</b>															
Chironomidae															
<i>Ablabesmyia</i> sp.	1	3	3	1	1	1	-	-	-	-	-	-	-	-	10
<i>Demicryptochironomus</i> sp.	-	2	-	-	-	-	1	-	-	-	-	-	-	-	3
<i>Dicrotendipes</i> sp.	-	2	6	1	2	1	1	-	-	-	-	-	1	-	14
<i>Nanocladius</i> sp.	-	-	-	-	-	1	-	1	-	-	-	-	1	-	3
<i>Paramerina</i> sp.	-	3	2	-	-	-	1	-	-	-	1	-	1	-	8
<i>Polypedilum</i> sp.	2	12	9	5	5	1	7	3	4	8	6	8	3	3	76
<i>Rheotanytarsus</i> sp.	-	-	-	-	-	-	-	-	5	1	2	1	-	-	9
<i>Tanytarsus</i> sp.	-	1	-	2	-	-	2	-	-	1	-	-	-	-	6
<i>Xenochironomus</i> sp.	-	9	10	1	3	-	2	-	7	3	3	2	1	2	43
Chironomidae gen. sp.	-	1	4	-	-	-	-	-	1	-	1	1	-	-	8
Ceratopogonidae															
<i>Bezzia</i> sp.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1
Total	3	47	38	11	16	6	19	94	65	29	21	13	9	8	379

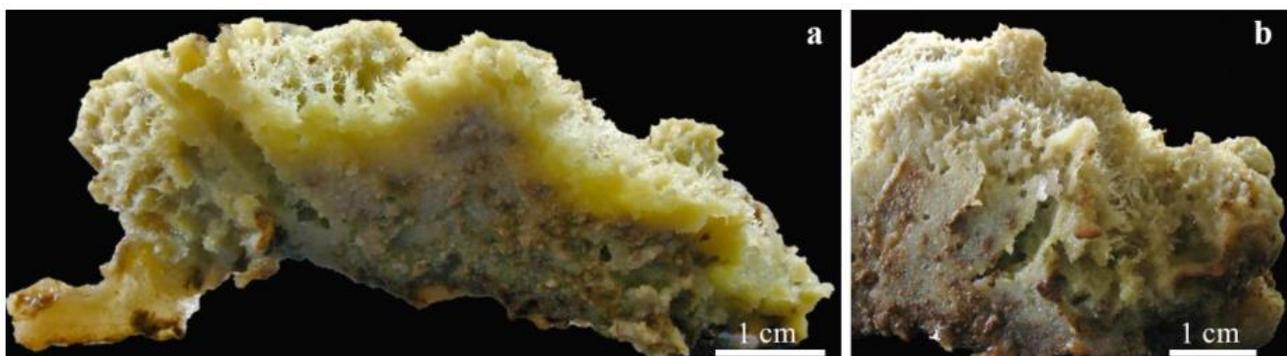
### 3.3. Gut Content Analysis

The gut content analysis of the four dominant taxa, i.e., *Ecnomus* spp., *A. meridiana*, *Polypedilum* sp., and *Xenochironomus* sp., respectively, showed a predominance of detritus, followed by diatoms, fine detritus material, rotifers, and sponge siliceous spicules (Figure 8c,d). Generally, the entire strongyle megascleres characterized by rounded tips were commonly found in the digestive tract, whereas fragments of these spicules rarely occurred. The percentage of spicules occurring in the gut contents of the representative dominant genera showed a wide range, i.e., *Ecnomus* spp. (70%; Trichoptera), *A. meridiana*

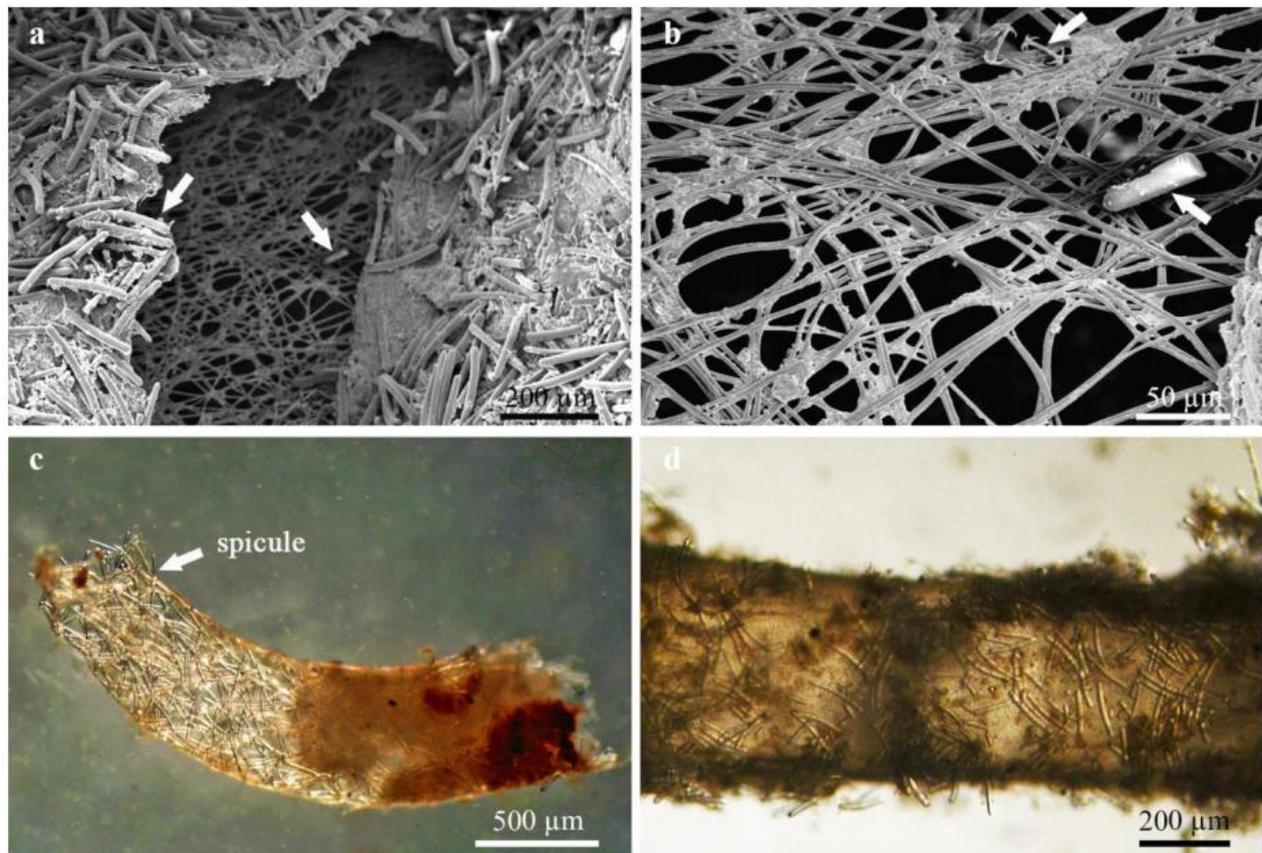
(56.6%; Trichoptera), *Xenochironomus* sp. (6.6%; Chironomidae), and *Polypedilum* sp. (6.6%; Chironomidae). In addition, megascleres and microscleres of sponges were also found in the chironomids *Ablabesmyia* sp. and *Paramerina* sp.



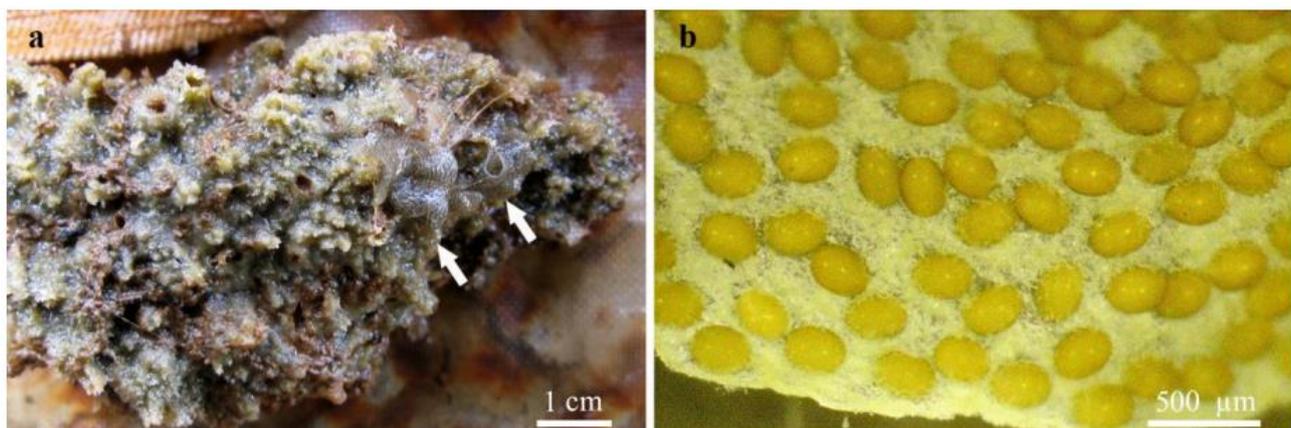
**Figure 3.** *Corvospongilla siamensis* (Porifera: Spongillida) in vivo settled on the raft artificial substrata of Pong River (Lower Mekong). (a) Outline of sponge conulose body surface with a variety of microhabitats at level of irregularly scattered crevices and ridges, and tube-like exhalant aquiferous openings (oscles). (b) Caddisfly larvae (*Amphipsyche meridiana*, Hydropsychidae, Trichoptera) crawling on a cavity-rich surface of a greenish sponge. (c,d) Chironomid larvae (Chironomidae, Diptera) burrowing in the inner sponge body.



**Figure 4.** *Corvospongilla siamensis* morphology (Pong River, Lower Mekong). (a,b) Heterogeneous microhabitat at the sponge irregular surface with ridges and large tube-like exhalant aquiferous aperture openings.

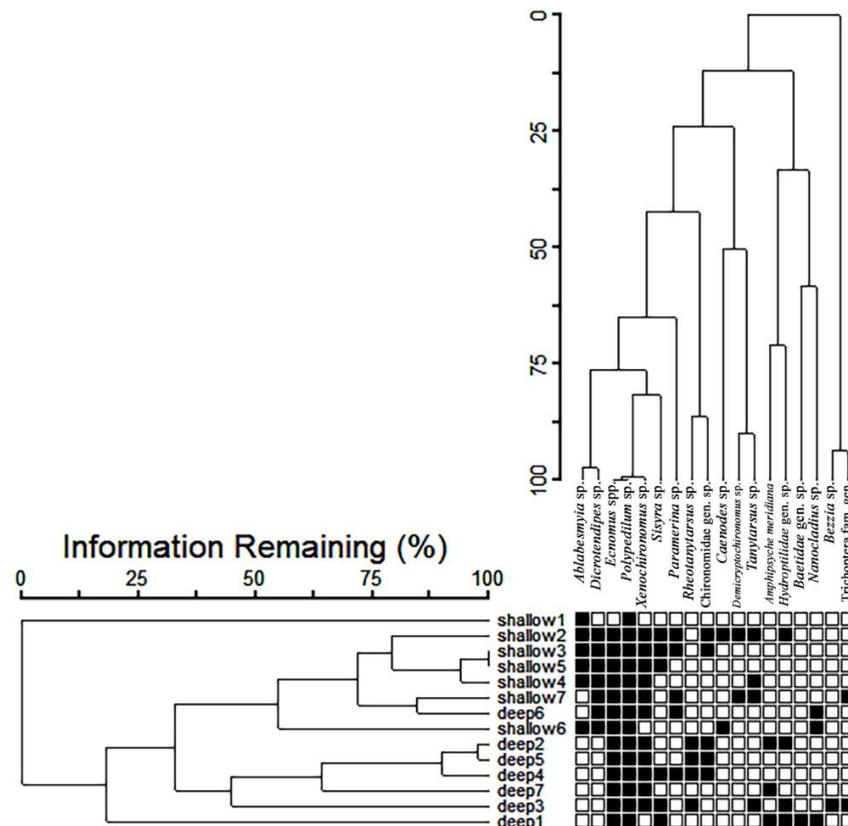


**Figure 5.** Sponge-dwelling caddisfly and non-biting midge larvae recycling siliceous spicules of *Corvospongilla siamensis* (Lower Mekong, Southeast Asia). (a,b) Pupae case of spongivorous *Amphipsyche meridiana* (Trichoptera, Hydropsychidae) reinforced by megascleres and microscleres (arrows, SEM) adhering to the silk network of the inner and outer cases. (c,d) Case of a chironomid larva (Chironomidae, Diptera) with a dense network of spicules (arrow, LM).

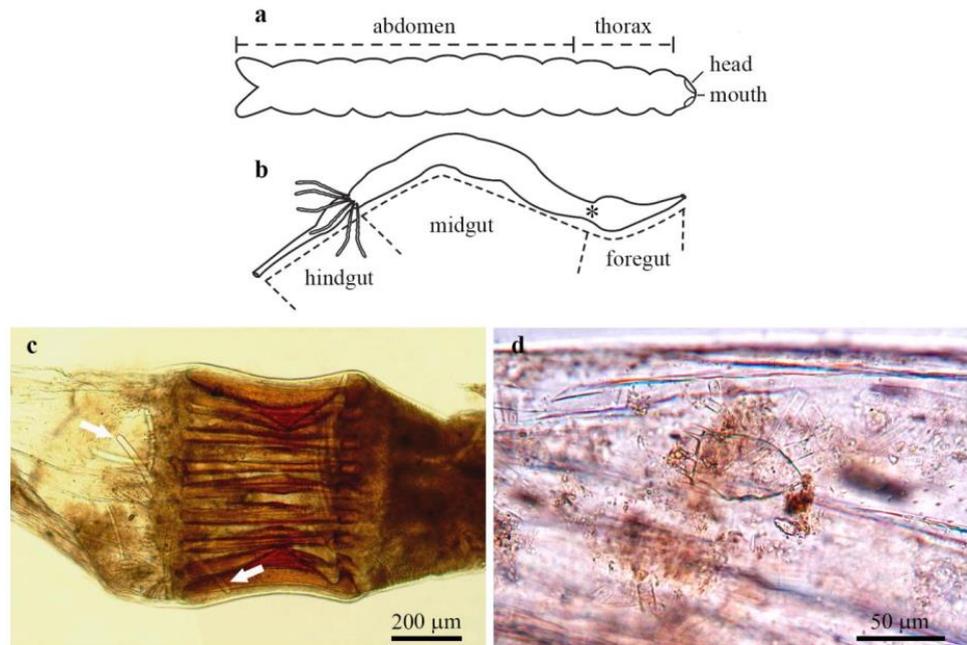


**Figure 6.** Eggs of *Povilla heardi* (Ephemeroptera, Polymitarcyidae) on *Corvospongilla siamensis* (Lower Mekong, Southeast Asia). (a) Egg jelly envelopes (arrows) on the irregular surface of a live sponge. (b) Eggs viewed by light microscopy.

The texture of the pupal case of the *A. meridiana* studied by an SEM showed that silk thread is a major component of the case, strengthened by two types of sponge spicules (microscleres, gemmuloscleres, and megascleres) on both the inner and outer layers of the cases (Figure 5a,b). The observations on the tubes of the chironomid larvae and pupae stages highlighted that both are made, in part, by sponge spicules (Figure 5c,d).



**Figure 7.** Occurrence and composition of sponge-dwelling larvae of aquatic insects associated with 14 samples of *Corvospongilla siamensis* (Pong River, Lower Mekong). One cluster comprised the shallow-water samples (0.3 m) and the other all but one of those from deep water (1 m).



**Figure 8.** *Amphipsyche meridiana* (Trichoptera, Hydropsychidae) associated with *Corvospongilla siamensis* (Lower Mekong, Southeast Asia). (a,b) Scheme of body and digestive tract of the sponge-eating caddisfly larva (adapted from Sangpradub and Giller [27]). The position of the proventriculus (\*) is indicated in (b). (c) Siliceous spicules with blunt tips (arrows) in the proventriculus. (d) Food debris in the midgut, i.e., diatoms and a rotifer.

## 4. Discussion

### 4.1. Freshwater Sponges as Microhabitat for Associated Insects

The data highlight that many insects can inhabit sponges in the Mekong River Basin (Table 1), matching the previous studies on sponge-dwelling entomofauna, reporting 10 orders of Insecta inhabiting almost 20 Spongillida species from all the biogeographic regions, except Antarctica (Table 2). The flourishing population of *Corvospongilla* (Figure 9) is a temporary microhabitat for the long-lasting larval and pupal stages of many insects, either at the body surface and canals, or within the extracellular matrix, suggesting that, also, other Spongillida species play the same functional role as the habitat-forming species in the same site, e.g., *Oncosclera asiatica* [28].

**Table 2.** Worldwide inventory of aquatic Insecta inhabiting Spongillida (Porifera) so far reported. Sponge species belong to families Lubomirskiidae, Metaniidae, Potamolepidae, and Spongillidae. Taxa and countries listed in alphabetical order. References listed in chronological order.

Insect Taxa	Sponge Taxa	References	Biogeographic Region/Country
Ephemeroptera (Baetidae, Caenidae, Polymitarcyidae) Neuroptera (Sisyridae) Trichoptera (Ecnomidae, Hydroptilidae, Hydropsychidae) Diptera (Ceratopogonidae, Chironomidae)	<i>Corvospongilla siamensis</i> <i>Eunapius carteri</i> <i>Radiospongilla crateriformis</i> <i>Spongilla alba</i> Spongillida fam. gen. sp.	Present paper, Annandale [29], Schröder [30]	<b>Oriental-Himalayan</b> Thailand; Borneo; India; Java; Philippines
—	<i>Spongilla alba</i> Spongillida fam. gen. sp.	Schröder [30], Mansell [31]	<b>Afrotropical</b> Madagascar; South Africa
Trichoptera (Hydroptilidae)	Spongillida fam. gen. sp.	Wells [32], Wells & Johanson [33], Forteath & Osborne [34], Forteath et al. [35]	<b>Australasian</b> Australia; New Zealand; New Caledonia; Tasmania
Diptera (Chironomidae) Ephemeroptera (Baetidae, Caenidae) Neuroptera (Sisyridae) Megaloptera (Sialidae) Trichoptera (Apataniidae, Brachycentridae, Ecnomidae, Hydropsychidae, Hydroptilidae, Leptoceridae, Limnephilidae, Polycentropodidae, Psychomyiidae)	<i>Ephydatia fluviatilis</i> <i>Ephydatia muelleri</i> <i>Eunapius fragilis</i> <i>Heteromeyenia</i> sp. <i>Lubomirskia baikalensis</i> <i>Spongilla lacustris</i> Spongillida fam. gen. sp.	Pavesi [36], Arndt [37], Rezvoi [38], Berg [39], Gaumont [40], Moretti & Corallini-Sorcetti [41], Konopacka & Socinski [42], Kamaltynov et al. [43], Weissmair & Mildner [44], Gugel [45], Corallini & Gaino [46,47], Gaino et al. [48], Wallace et al. [49], Weinberg et al. [50], Loru et al. [51], Schiffels [52], Sokolova & Palatov [53], Palatov & Sokolova [54], Zvereva et al. [55], Ostrovsky [56]	<b>Palaeartic</b> Algeria; Austria; China; East Siberia; Denmark; England; France; Germany; Italy; Japan; Mongolia; Poland; Russia; Scandinavia; Serbia; Spain
Diptera (Chironomidae, Simuliidae) Coleoptera (Dytiscidae) Ephemeroptera (Polymitarcyidae, Tricorythidae) Hemiptera (Belostomatidae) Lepidoptera (Pyralidae) Neuroptera (Sisyridae) Odonata (Coenagrionidae, Libellulidae) Trichoptera (Hydropsychidae, Polycentropodidae)	<i>Acalle recurvata</i> <i>Drulia uruguayensis</i> <i>Corvoheteromeyenia australis</i> <i>Corvospongilla seckti</i> <i>Heteromeyenia cristalina</i> <i>Metania spinata</i> <i>Metania reticulata</i> <i>Oncosclera navicella</i> <i>Oncosclera spinifera</i> <i>Radiospongilla inesi</i> <i>Trochospongilla paulula</i>	Melão & Rocha [57], Roque et al. [58], Fusari et al. [59–62], Clavier et al. [63], Hamada et al. [64], Bowles [65], Ardila-Camacho & Martins [66], Assmar & Salles [67], Fisher et al. [68], da Silva Fernandes [69]	<b>Neotropical</b> Argentina; Belize; Bolivia; Brazil; Chile; Cuba; French Guiana; Guatemala; Guyana; Honduras; Mexico; Panama; Paraguay; Peru; Suriname; Uruguay; Venezuela
Neuroptera (Sisyridae) Megaloptera Trichoptera (Leptoceridae)	<i>Eunapius fragilis</i> <i>Ephydatia muelleri</i> <i>Ephydatia fluviatilis</i> <i>Eunapius igloviiformis</i> <i>Dosilia radiospiculata</i> <i>Heteromeyenia argirosperma</i> <i>Heteromeyenia baileyi</i> <i>Heteromeyenia repens</i> (as <i>H. baileyi</i> ) <i>Rackiella ryderi</i> (as <i>Heteromeyenia ryderi</i> ) <i>Radiospongilla crateriformis</i> <i>Spongilla lacustris</i> <i>Ephydatia robusta</i> (as <i>Meyenia subdivisa</i> ) <i>Trochospongilla horrida</i> <i>Trochospongilla leidii</i> (as <i>T. leidyi</i> ) <i>Trochospongilla muelleri</i> <i>Trochospongilla pennsylvanica</i> (as <i>Tubella pennsylvanica</i> )	Brown [70], Parfin & Gurney [71], Roback [72], Heiman & Knight [73], Poirrier [74], Poirrier & Arceneaux [75], Resh [76], Pennak [77], Matteson & Jacobi [78], Stoakes et al. [79], Clark [80], Pupedis [81], Whitlock & Morse [82], Bowles [65,83], Cover & Resh [84], Rothfuss & Heilveil [85], Fisher et al. [68], Bowles & Courtney [86]	<b>Nearctic</b> United States of America Canada



**Figure 9.** Dense assemblage of freshwater sponges with massive, irregular growth form as engineering species on farm nets in the Pong River (Lower Mekong, Southeast Asia). (a) The red area outlining the body of *Corvospongilla siamensis* is  $\sim 800$  cm<sup>2</sup>. (b) Living *Corvospongilla* sp. with associated algae. (c) Dry freshwater sponges on net.

#### 4.2. Associated Insects and Environmental Conditions

Even though the dissolved oxygen (DO) was significantly different between the two depths, it did not seem to affect the sponge-dwelling insect assemblage structure, i.e., the relative abundance and taxonomic richness.

The flow-velocity data also support the fact that the DO is not a limiting factor to the sponges' inhabitants in the study site, which is located near the outlet of the dam. This could be due to both the oxygen contribution from the sponge algal symbiont (endocellular) and the hydrodynamic condition of the sponge microenvironment. Indeed, the sponges' pumping activity, involving conspicuous amounts of water, creates a microcirculation, both within the sponge body (inner canals and cavities) and at its irregular surface (inhalant openings with incurrent and oscules with outcurrent water), matching the previous data [87,88].

Although the composition of the associated aquatic insects between the shallow and deep zones is similar in sharing 11 taxa, it seems that the flow-velocity values could be a factor that affects the number of *Amphipsyche meridiana*, inhabiting only two samples in the deeper zone, while the higher values may favor these net-spinning larvae. In addition, the division between the "shallow and deeper parts" of the cluster differs in the greater abundance of Trichoptera, from the genus *Ecnomus* and *Amphipsyche*, requiring higher oxygen concentrations than the chironomids, which is also confirmed by the environmental data and could be the consequence of the higher flow velocity.

The colonization of *Polypedilum* sp. in all the samples indicates that *Corvospongilla* is a favorable habitat matching the known wide habitat range of this chironomid genus [24,69]. The present data, reporting Chironomidae larvae fairly commonly in the sponges from

both the two shallow zones, match those of the Chironomidae [48,61,69], which is well represented in the Pong River's soft bottom, ranging from pristine to impacted water quality conditions [89]. Further in-depth analyses of the taxonomy and diversity of the chironomid larvae in the Pong River will be essential to clarify the sponge–midge interactions.

The present data also corroborate those of Gugel [45], who reported that the high larvae number of *Ecnomus tenellus* (Rambur, 1842) and *Hydropsyche* sp. occurring on European sponges was higher than their occurrence independently from sponges. The results confirm that sponges represent a favorable habitat for these larvae, as reported also by Annandale [29,90] for other hydrographic basins of the Asian tropics.

#### 4.3. Aquatic Insect Abundance, Sponge Size and Body Architecture

The high number of sponge-dwelling aquatic insects in this study occurred in a silty riverbed with a dense population of long-living, massive sponges, characterized by a 3D body architecture, rich in large inner canals/cavities and exhalant apertures (Figures 3a,4a,b and 9), matching the investigations on marine sponge canals diameter and body morphology inhabited by invertebrates [91,92]. The sponge body architecture, rich in cavities, seems to provide a suitable space for some aquatic insects to segregate and avoid predators, as suggested also by the high density of freshwater African *A. meridiana* in vesicular rocks (i.e., volcanic rock pitted with many cavities/vesicles at its surface and inside), which was about 10 times higher than those of non-vesicular rocks [93].

Only the biomass and numbers of three species of amphipods inhabiting sponges are known to be correlated with the weight of a Baikalian sponge species, according to Kamal'tynov et al. [43], but the other fauna including insects showed no correlation.

#### 4.4. Interspecific Relationships between Aquatic Insects and Sponges

Several genera of Insecta have been recognized as obligatory associated with sponges, i.e., *Ceraclea* (Trichoptera), *Demeijerea*, *Oukuriella*, *Xenochironomus* (Chironomidae), *Climacea*, and *Sisyra* (Neuroptera) [46,47,53,54,60,69,72,76,82,94] (see Table 2). Spongivory is, however, rare, probably due to the production of sponge toxins and repellents [13,95]. Sisyridae larvae are well known as obligate predators (spongivory) of freshwater sponges [71,76,84,96].

In the present study, the type of sponge–insect relationship ranges from incidental to obligate. The larvae of *Sisyra* and *Xenochironomus* we discovered in sponges had never been found in the Pong River's macroinvertebrate community [89,97,98]. Weissmair and Warning [99] stated that the *Sisyra* adults need rich riparian habitats, which occur rarely along the Pong River. This datum reinforces the opinion that the larvae of these taxa are strictly associated with freshwater sponges.

Little information has been published in Asia on the *Sisyra* biology, ecology, and phylogeny [100,101]. Only two species, *Sisyra indica* Needham, 1909, and *Sisyra maculata* Monserrat, 1981, are known from Thailand [83,84,102,103], suggesting further studies on neglected neuropterans and *Xenochironomus* species and their life cycles in tropical sponges of Asia would be worthwhile.

#### 4.5. Sponges and Gut Content of Their Associated Aquatic Insects

Roback [72] reported that the digestive tracts of caddisfly larvae belonging to the genus *Athripsodes* were filled with debris and sponge spicules. These larvae ingest the sponge tissues and do not merely use them as an occasional substrate or case-building material. In this study, the gut contents of the caddisfly *A. meridiana* larvae (Figure 8a–d), showing high percentages of diatoms, may indicate that these net-spinning caddisfly larvae are filtering collectors. Our results agree with Boon [104], who concluded that the well-developed gastric mill plates of *A. meridiana* are ideal for crushing diatoms. Although it is known that sponges are not edible for most animals, due to their chemical deterrence, with hard skeletal components and bioactive compounds, the occurrence of many spicules in the digestive tracts may also suggest that *A. meridiana* feeds on the tissues of the sponge when moving on its surface, which matches the previous reports [47,72]. Entire siliceous

strongyle spicules in the digestive tract could improve the grinding or filtering of foods, as suggested by not breaking down—even passing—the proventriculus, which consists of large tooth plates ranging from 533 to 656  $\mu\text{m}$  in length (Figure 8c). Moreover, it seems that the spicules of *C. siamensis*, with typical blunt tips, cannot create a risk of damage to the gut wall of spongivore insects, contrasting with the condition of caddisfly *Ceraclea fulva* (Rambur, 1842), displaying an abnormally thick peritrophic membrane shielding its soft internal tissues from ingested spicules, with acute tips, of the sponge *Ephydatia fluviatilis* [46,47].

As for the pupal case of *A. meridiana*, an SEM analysis showed that sponge spicules were interwoven in the case wall, which is similar to those observed in *C. fulva*. So, these spicules seem to be useful material to reinforce the cases of the caddisfly larvae (Figure 5a,b). The chironomid larvae and pupae also utilized the sponge spicules for constructing their tubes and cases in the Pong River (Figure 5c,d), thus corroborating previous data on the occurrence of sponge spicules in the gut [94].

#### 4.6. Biodiversity and Conservation Perspectives

The results of this study expand our knowledge of freshwater sponges in Southeast Asia, which is considered a biodiversity hotspot, highlighted by the 3007 new species of vascular plants, fishes, amphibians, reptiles, birds, and mammals discovered in the Greater Mekong region between 2007 and 2020 [105,106]. The description of three new Spongillida species and a new record in Thailand and Vietnam [20,28,107,108], and ongoing discoveries in Thailand (Ruengsawang unpublished data), indicate that the sponges inhabiting rivers, lakes, and wetlands in this region remain unexplored and are underestimated in their biodiversity. Environmental impacts on freshwater sponges have been reported, i.e., temperature, heavy metal contamination [109], and habitat change, as one of biodiversity loss drivers in Southeast Asia [110]. Therefore, a diversity assessment of this neglected fauna is urgently needed.

In the case of the Pong River, the biofouling by sponges in fish culture, resulting in clogging of the nets and decreased water circulation, was frequently found in the previous and current studies [19]. Fish farmers lift their cage nets from the water to dry and remove sponges for maintenance after fish harvesting (Figures 9 and 10). Skin irritation caused by sponge spicules was also reported by the farmers. The advantages and disadvantages of sponges biofouling in the fish culture in the Pong River need further research to fully understand to demonstrate the ecological value of these animals.



**Figure 10.** Fish cage maintenance after harvesting in the Pong River. (a) Fisherman with dried nets. (b,c) Freshwater sponges encrusting on farm nets.

From the conservation aspect, it is clear that freshwater sponges play an important role in aquatic ecosystems as biofilters, through their filter-feeding and water-pumping activities and are vulnerable habitats for aquatic insects. The opportunistic insect from this study, *A. meridiana*, adopts a variety of strategies to colonize and to use sponges as a microhabitat, which agrees with Boon [93]. Despite freshwater sponges not being threatened globally [1,10,109,111], the conservation and monitoring of freshwater sponges in tropical Asia are needed to understand their status.

### Synthesis

The data indicate that, worldwide, freshwater sponges perform functional roles as ecosystem engineers, sensu Jones et al. [112]. They supply to insects: (a) persistent heterogeneous substrata and microclimatic conditions related to the sponges' massive to arborescent growth form and their physiological performance, (b) ecological refugia and breeding/nursery grounds as a sheltering microhabitat for settling and the development of eggs, (c) food source for detritivores by the continuous water-flow, trapping particulate organic matter, as a foraging area, for eating on sponge-associated algae, and as prey for predatory species on other hosted insects and a plethora of other invertebrates, (d) the morpho-functional role of the siliceous spicules to strengthen the larval insect's digestive system and the cases of larvae and pupae, (e) protection against predators, (f) shelter for withstanding floods, high siltation, and desiccation (dry season) during water level fluctuations. Despite the sponges' functional role as biofilters, on occasion, local people consider these animals responsible for economic damage, e.g., on the health of fish in farming cages along the Lower Mekong River. In agreement with the restoration science proposed by Ormerod [113], we suggest that sponge farming in inland water could be an effective nature-based solution to reduce biodiversity loss, as a management tool, by enhancing the water quality. This is particularly relevant in tropical freshwater to support bioremediation projects in restoring the environmental value, also in giving economic benefits to the local people. Another key point could be a targeted action on the people's environmental education in order to raise awareness of freshwater bioresources conservation.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14110911/s1>; Table S1: Mean values ( $\pm$ SD) for physicochemical parameters during the dry season (northeast monsoon) at two shallow water depths inhabited by a flourishing population of *Corvospongilla siamensis* and its associated insects in the Pong River (Lower Mekong, Thailand).

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