Diversity, distribution and habitat associations of aquatic beetles (Order Coleoptera) in Chambok, southwest Cambodia

DOEURK Bros[1,2,3,*], CHHORN Soksan[1,2,4], SIN Sopha[1,2], PHAUK Sophany[2] & SOR Ratha[4,5]

[1] Centre for Biodiversity Conservation, Royal University of Phnom, Russian Confederation Blvd, Phnom Penh 12156, Cambodia.

[2] Cambodian Entomology Initiatives, Department of Biology, Faculty of Science, Royal University of Phnom Penh, Russian Confederation Blvd, Phnom Penh 12156, Cambodia.

[3] Malaria Molecular Epidemiology Unit, Institut Pasteur du Cambodge, 5 Monivong Boulevard, P.O. Box 983, Phnom Penh 12201, Cambodia

[4] Wonders of the Mekong Project, c/o Inland Fisheries Research and Development Institute, 186 Preah Norodom Boulevard, P.O. Box 582, Phnom Penh 12300, Cambodia.

[5] International Relations, Research and Collaborations & Graduate School, Chea Sim University of Kamchaymear, 157 Preah Norodom Boulevard, Khan Chamkarmon, Phnom Penh 12300, Cambodia.

* Corresponding author. Email dourkbros@gmail.com

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មូលន័យសង្ខេប

យើងបានធ្វើការអង្កេតទៅលើនានាភាព របាយ និងទំនាក់ទំនងជាមួយទីជម្រករបស់សត្វល្អិតទឹកស្លាបរឹង នៅឃុំចំបក់ ខេត្តកំពង់ស្ពឺ ភាគនិរតីនៃប្រទេសកម្ពុជា។ សំណាកសិក្សាត្រូវបានប្រមូលចាប់ពីខែសីហា ដល់ខែកញ្ញា ឆ្នាំ២០១៨ នៅទីតាំងចំនួន១០កន្លែង នៅក្នុងឃុំនេះ។ ជាលទ្ធផល យើងប្រមូលបានសត្វល្អិតស្លាបរឹងចំនួន ១៥២ក្បាល ត្រូវនឹង៩អំបូរ ១០ពួក និង២២ប្រភេទ។ អំបូរ Scirtidae ត្រូវបានចាប់ក្នុងបរិមាណច្រើនជាងគេចេញពីទីតាំងទាំង១០កន្លែង ដែលអាចបង្ហាញបានថា អំបូរនេះមាននានាភាពច្រើន និងរស់នៅតាមទីកន្លែងផ្សេងៗគ្នា។ Elmidae ជាអំបូរដែលចាប់បានច្រើនលំដាប់ទី២ ហើយបន្ទាប់មកគឺអំបូរ Dryopidae, Hydrophilidae, Limnichidae, Hydraenidae, Dytiscidae, Gyrinidae និងអំបូរ Chrysomelidae។ ការវិភាគតាម Non-metric multidimensional scaling analysis បានបង្ហាញថា អំបូរខ្លះអាស្រ័យលើទីជម្រកផ្សេងៗគ្នា។ ឧទាហរណ៍ អំបូរ Scirtidae មានទំនាក់ទំនងជាវិជ្ជមានជាមួយគម្របព្រៃឈើ ប៉ុន្តែវាមានទំនាក់ទំនងជាអវិជ្ជមានជាមួយនឹងការប្រើប្រាស់ដីកសិកម្ម។ ផ្ទុយមកវិញ អំបូរ Limnichidae មានទំនាក់ទំនងជាវិជ្ជមានជាមួយរយៈកម្ពស់ និងកម្រិតហូរច្រោះ នៅពេលដែលអំបូរ Dytiscidae មានទំនាក់ទំនងជាវិជ្ជមានជាមួយនឹងបរិមាណថ្មតូចៗក្នុងទីជម្រកនៅតាមដងអូរ ប៉ុន្តែវាមានទំនាក់ទំនងជាអវិជ្ជមានជាមួយនឹងជម្រៅទឹកនិងបរិមាណថ្មធំៗក្នុងទីជម្រក។ លទ្ធផលនៃការសិក្សានេះបង្ហាញថា វត្តមាននៃកូនញាស់និងសត្វពេញវ័យរបស់អំបូរទាំងនេះ ប្រែប្រួលទៅតាមប្រភេទទីជម្រកតូចៗជាក់លាក់ (microhabitats) ដែលជាកត្តាគាំទ្រឱ្យមានបណ្តុំប្រភេទខុសៗគ្នានៃពពួកសត្វល្អិតទឹកស្លាបរឹង។ លទ្ធផលទាំងនេះអាចផ្តល់ជាមូលដ្ឋានដ៏មានប្រយោជន៍សម្រាប់ការសិក្សាស្រាវជ្រាវអំពីសត្វល្អិតទឹកស្លាបរឹងនៅតំបន់ផ្សេងៗនៃប្រទេសកម្ពុជា និងនៅពេលអនាគត។ តែទោះយ៉ាងណាក្តី យើងស្នើឱ្យមានការសិក្សាជាបន្តទៀត ដើម្បីបញ្ជាក់បន្ថែមនិងពង្រីកលទ្ធផលនៃការស្រាវជ្រាវរបស់យើង។

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Abstract

We investigated the diversity, distribution and habitat associations of aquatic beetles in Chambok Commune of Kampong Speu Province, southwest Cambodia. Sampling at ten locations within the commune in August–September 2018 resulted in the collection of 152 individuals representing 22 morphospecies, ten genera and nine families. Scirtidae was the most abundant family across the ten sampling sites, which may reflect its greater diversity and wider distribution as a group. Elmidae was the second most common family, followed by Dryopidae, Hydrophilidae, Limnichidae, Hydraenidae, Dytiscidae, Gyrinidae and Chrysomelidae. Non-metric multidimensional scaling analysis revealed that some families differed in their habitat associations. For example, Scirtidae was positively associated with forest and canopy cover, but negatively associated with agricultural land use. In contrast, Limnichidae was positively correlated with altitude and erosion levels, whereas Dytiscidae was positively associated with stream substrates characterised by small stones, but negatively associated with water depth and substrates characterised by large stones. Our results suggest that the occurrence of larvae and adults of these families varies in response to different types of microhabitats which consequently support different species assemblages of aquatic beetles. While further investigations are desirable to confirm and extend these findings, our study nonetheless provides a useful baseline for future research efforts on aquatic beetles in Cambodia.

Keywords Beetles, habitat characteristics, Shannon Index, species diversity, non-metric multidimensional scaling.

Introduction

Beetles (order Coleoptera) are the largest insect order with approximately 400,000 species currently described and are estimated to comprise ≈1.1 million species worldwide (Oberprieler *et al*., 2007). However, less than 2% of these are adapted to aquatic life (Jäch & Balke, 2008). Aquatic and riparian beetles dwell in various habitats including wetland, lakes, streams and terrestrial habitats associated with water (Balke *et al*., 2014). They are regarded as aquatic beetles because either the larvae or adults (or both) occur in water (Bouchard, 2004). The larvae of aquatic beetles are characterised by a sclerotized head capsule, three pairs of legs and frequent presence of swimming hairs around the body (Bouchard, 2004). The adults are characterised by a strongly-sclerotized body with the forewings hardened into elytra, which serve to protect the more delicate hind wings, as well as the dorsal surface of the last two thoracic segments (pterothorax) and abdomen (Jäch & Balke, 2008). The size of the latter ranges from 1 mm to 3 cm depending on the family and species and the size of larvae can exceed that of adults (Balke *et al*., 2014).

Aquatic beetles can be found in various freshwater habitats including rivers, creeks, springs, lakes, ditches, puddles, lithotelmata, phytotelmata, seepages and groundwater (Freitag, 2015), although they are most diverse in lentic habitats such as marshes and pond margins (Bouchard, 2004). Their occurrence typically varies in response to microhabitat characteristics including particulate organic matter, algae, zooplankton and abiotic factors (i.e., temperature, substrate composition and altitudinal ranges) (Braun *et al*., 2018). For instance, they are greatly influenced by water quality and occur at low abundance when primary habitats are removed (Jäch & Balke, 2008). As a consequence, some aquatic beetles have been used as indicators for ecological diversity and habitat characteristics (Shailendra *et al*., 2013).

Aquatic beetles are among the most common aquatic insects on the planet, and are only exceeded by true flies (Order Diptera) in the number of taxa (though only as larvae). Over 13,000 species of aquatic beetles are presently recognised (Short, 2018) and many new species are described each year. From 2006 and 2010 for instance, nearly 200 species and five genera of Hydrophilidae were described, although as almost a dozen genera were synonymized during the same period, the number of valid genera actually decreased (Short & Fikáček, 2013). Thirty-nine species of aquatic Polyphaga have been reported in Cambodia to date (Hansen, 1998; Short & Hebauer, 2006; Short & Fikáček, 2011; Freitag *et al*., 2018).

Few studies have been conducted on aquatic beetles in Cambodia to our knowledge. Existing reports include records of 21 taxa from southwest Cambodia (Freitag *et al*., 2018), four aquatic beetle families in urban ponds in Phnom Penh (Chhy *et al*., 2019) and nine coleopteran taxa from the lower Mekong Basin region in Cambodia (Sor *et al*., 2017). The aim of our study was to investigate the diversity, distribution and habitat associations of aquatic beetles in a freshwater stream in southwest Cambodia and more broadly to improve understanding of aquatic beetle biodiversity and ecology in the country.

Table 1 Locations, elevations and dates of sampling in Chambok commune.

Site	Longitude	Latitude	Elevation (m)
S1	11°21'25.1"N	104°06'10.8"E	425 [1]
S2	11°21'27.7"N	104°06'14.3"E	394 [1]
S3	11°21'31.3"N	104°06'12.3"E	391 [1]
S4	11°21'53.4"N	104°06'12.4"E	293 [2]
S5	11°21'59.0"N	104°06'17.3"E	241 [2]
S6	11°22'03.9"N	104°06'26.7"E	197 [2]
S7	11°22'07.1"N	104°06'35.6"E	150 [3]
S8	11°22'14.0"N	104°06'42.7"E	133 [3]
S9	11°22'24.6"N	104°06'44.8"E	117 [3]
S10	11°22'30.7"N	104°06'49.9"E	116 [3]

Notes: Sampled with hand nets on 13[1], 14[2] and 15[3] August 2018. Artificial substrate traps were retrieved from sites on 11 September 2018.

Methods

Study site

We sampled aquatic beetles in August and September 2018 along a freshwater stream in a community-based ecotourism site located in Chambok Commune, Phnom Sruoch District, Kampong Speu Province. The study stream originates from a freshwater reservoir in the upper part of Kirirom National Park, a few kilometres from the Kirirom Hillside Resort. Ten sites (S01–S10) were sampled along the stream in several habitat types at different elevations (Table 1, Fig. 1). The distance between the upper and lowermost sampling sites (S01 & S10) was approximately three kilometres.

Field sampling

Two methods were employed for sampling aquatic beetles, hand nets and artificial substrate traps. Each sampling site was sampled with three replicates situated in different parts (left, right & middle) of the stream, resulting in a total of 30 samples. Hand nets with a mesh size 0.1 mm were employed for ≈30 minutes at each site to sample aquatic beetles along the stream-shore in different microhabitats including bottom gravel, rock surfaces, submerged root packs, submerged woods and other bottom substrates. After rinsing each net sample with water, aquatic beetles were extracted using soft forceps and transferred to labelled vials containing 95% ethanol. The substrate of our artificial traps comprised rocks and naturally occurring substrate in the area and were deployed at each site for ≈30 days. As with our hand net samples, aquatic beetles in each trap were extracted with soft forceps and transferred into labelled vials containing 95% ethanol.

The adult specimens were identified in the laboratory to morphospecies and larvae were identified to family level based on Balke *et al*. (2014), MRC (2006) and an unpublished identification key for freshwater macroinvertebrates in Southeast Asia. These identifications were subsequently reviewed in part by a specialist on Southeast Asian aquatic beetles (Dr Hendrik Freitag, Ateneo de Manila University, Philippines).

We used a multi-parameter meter with GPS (Hanna Instruments, Rhode Island, USA) to record the altitude and water temperature of each sampling site. Several habitat characteristics, including stream width, water depth, erosion and substrate composition (large stones [>250 mm], medium stones [64–250 mm], small stones [2–64 mm], sand & silt/clay) were also recorded (Annex 1). Erosion measurements were based on the loss of the soil mass of bank channels over time. Three categories were recognised, with a value of zero (0) indicating no erosion, a value of one (1) indicating <30% erosion and value of two (2) indicating >30% erosion (Raven *et al*., 1998; Parsons *et al*., 2002). Additionally, percentage land cover (i.e. forest, agriculture, residential areas, orchards, bamboo & shade/canopy) was recorded in the vicinity of each sampling site following the Australian River Assessment System (Parsons *et al*., 2002) and River Habitat Survey protocols (Raven *et al*., 1998).

Statistical analyses

We employed taxonomic richness, taxonomic abundance and the Shannon diversity (H) index to characterize the diversity of aquatic beetles at our study sites using the 'vegan' package of Oksanen *et al*. (2015). Simple linear regressions were initially performed to test the relationship between environmental variables and aquatic beetle families. Significantly related variables were retained in subsequent analysis. Non-metric multidimensional scaling (NMDS) was employed to visualize the associations between these variables and aquatic beetle families and stress scores were used to determine the reliability of NMDS results (Clarke, 1993). Correlation coefficients were employed to assess the statistical power of our results and p values < 0.05 were considered significant. All analyses were performed in R v.4.0.3 (R Core Team, 2020).

Fig. 1 Sampling sites (S1–S10) along study stream in Chambok Commune, Kampong Speu Province, southwest Cambodia.

Table 2 Abundance and occurrence of aquatic beetle morphospecies at sampling sites in Chambok Commune.

No	Family / Morphospecies	Abundance	Occurrence (%)	Sampling Sites
Hydrophilidae				
1	*Pelthydrus* sp.	1	10	S09
2	*Pelthydrus* sp.1	2	20	S06, S09
3	*Pelthydrus vitalisi*	3	20	S09, S10
4	*Agraphydrus* sp.	1	10	S07
5	Hydrophilidae larvae	3	20	S05, S10
Hydaenidae				
6	*Hydraena* sp.1	2	10	S10
7	*Hydraena* sp.2	1	10	S10
8	*Hydraena* sp.3	3	20	S09, S10
9	*Hydraena* sp.4	3	30	S07, S09, S10
Elmidae				
10	*Grouvellinus* sp.2	1	10	S01
11	Elmidae larvae	12	60	S01, S04–S06, S08, S09
Dryopidae				
12	*Elmomorphus* sp.	11	50	S02, S03, S05, S08, S10
Dytiscidae				
13	*Neptosternus hydaticoides*	1	10	S10
14	Dytiscidae larvae	6	20	S03, S09
Gyrinidae				
15	*Patrus* sp.1	3	10	S06
Limnichidae				
16	Limnichidae sp.	4	30	S01, S03, S05
17	*Caccothryptus* sp.	2	20	S02-S03
18	*Throscinus* sp.	2	10	S03
19	*Byrrhinus* sp.	1	10	S03
Scirtidae				
20	Scirtidae larvae	87	100	S01–S10
21	Scirtidae adults	2	20	S03, S04
Chrysomelidae				
22	Chrysomelidae larvae	1	10	S06

Results

We recorded 152 individuals belonging to 22 morphospecies, ten genera and nine families in Chambok (Table 2). Among these, Scirtidae was the most common family and found at all ten sites with a total of 89 individuals (Figs. 2 & 3). This was followed in decreasing order by Elmidae (six sites, 13 individuals), Dryopidae (five sites, 11 individuals), Hydrophilidae (five sites, ten individuals), Limnichidae (four sites, nine individuals), Hydraenidae (three sites, nine individuals) and Dytiscidae (three sites, seven individuals) (Figs. 2 & 3). Gyrinidae and Chrysomelidae were the least common families and occurred at only one site with three and one individuals, respectively. Across our sampling sites, the taxonomic richness of aquatic beetles ranged from one to ten taxa (5 ± 2.7), whereas abundance ranged from five to 36 individuals (15.2 ± 9.9) and Shannon diversity values from 0 to 1.5 (1.1 ± 0.5).

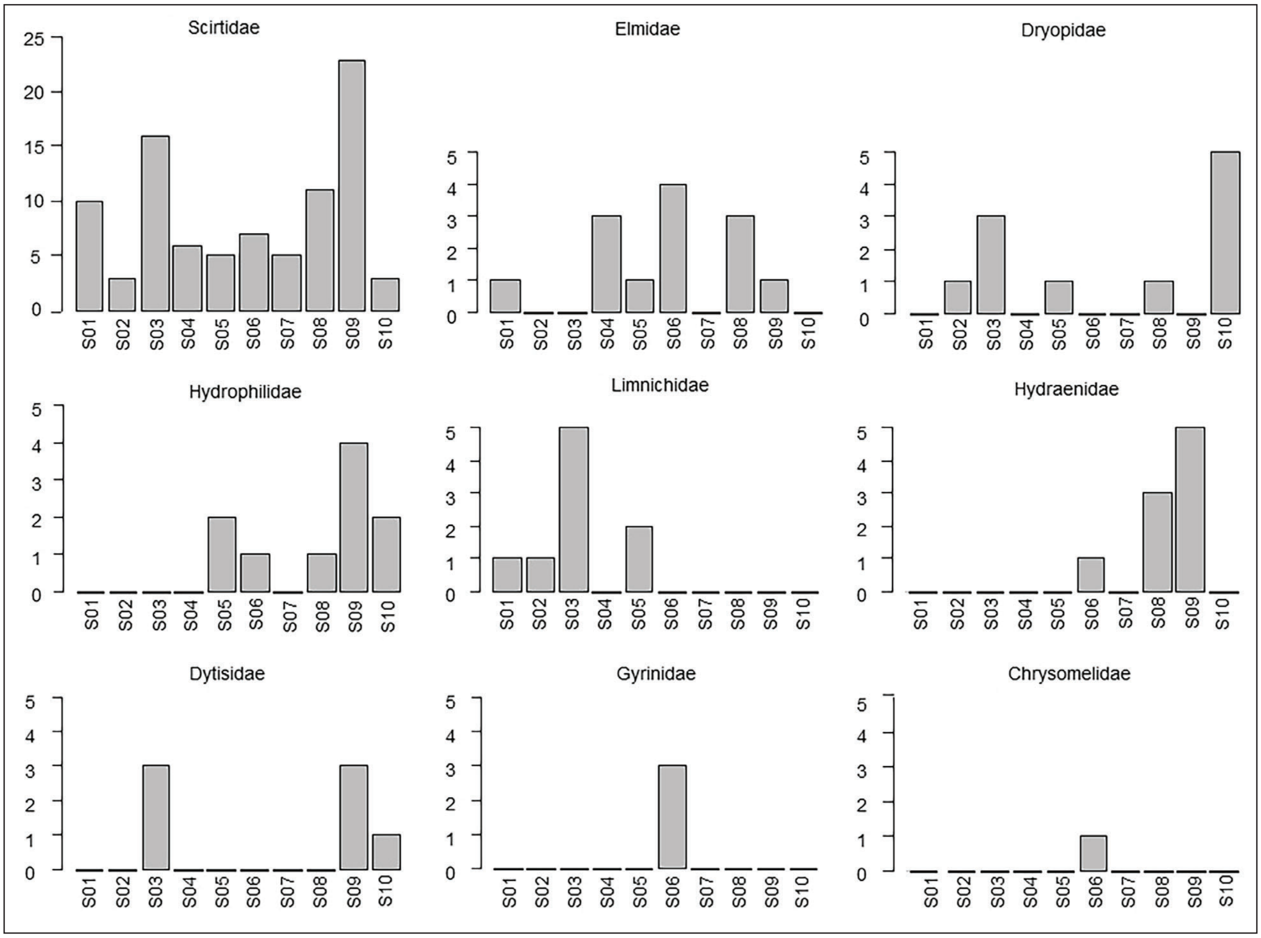

Fig. 2 Abundance of aquatic beetle families by sampling site in Chambok Commune.

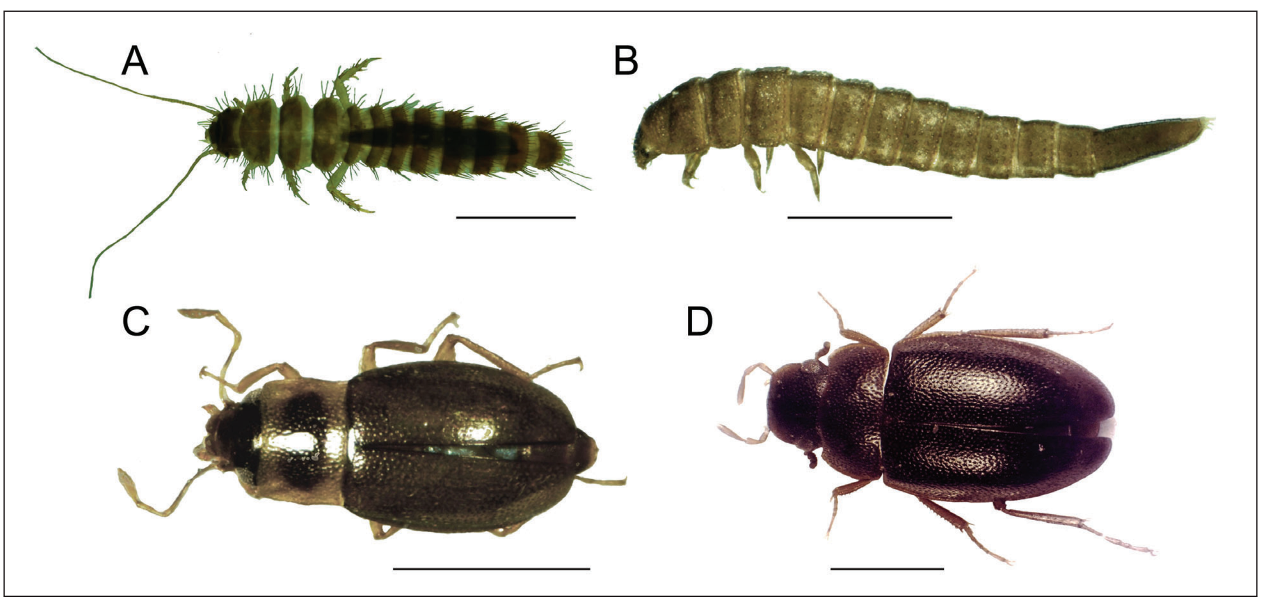

Fig. 3 Selected aquatic beetle families recorded during the study: A) Scirtidae larva, B) Elmidae larva, C) Adult *Hydraena* (Hydraenidae), D) Adult *Pelthydrus* (Hydrophilidae). Scale bars = 1 mm.

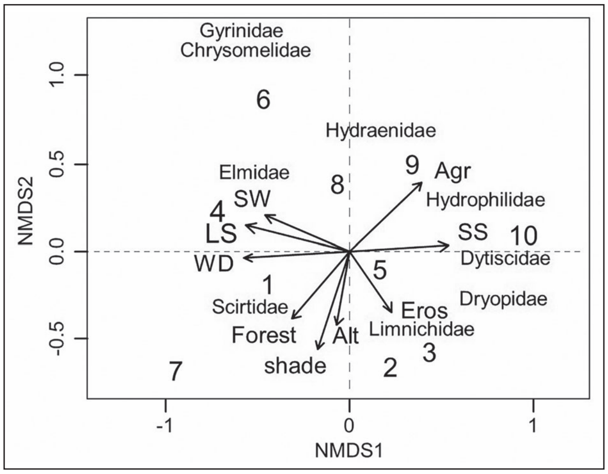

Fig. 4 Non-metric multidimensional scaling plot illustrating the association between sampling sites (1–10), aquatic beetle families and environment variables (WD=water depth, Alt: =altitude, Eros=erosion, SS=small stones, Agr=agriculture, LS=large stones, SW=stream width).

Habitat association of aquatic beetle families

Our linear regressions indicated nine environment variables were significantly correlated with aquatic beetle family ordination scores ($p < 0.05$), namely altitude, water depth, stream width, erosion levels, forest cover, agricultural land use and shade and the quantity of large and small stones in stream substrates. The result of our NMDS (stress score = 0.08) depicts these relationships (Fig. 4). Scirtidae was positively associated with forest cover and shade, but negatively with agricultural land use. Limnichidae was positively associated with altitude and erosion levels, whereas Dytiscidae was positively associated with small stone substrates, but negatively associated with large stone substrates and water depth. Hydrophilidae was positively associated with agricultural land use and negatively associated with forest cover, whereas Elmidae was positively associated with stream width, water depth and large stone substrates. The remaining families (Gyrinidae, Chrysomelidae & Hydraenidae) were not clearly associated with any of the environmental variables we sampled.

Discussion

Existing literature on invertebrates in Chambok Commune includes a species list of 21 aquatic beetle taxa (Freitag *et al*., 2018), one study on mayflies (Ephemeroptera) (Chhorn *et al*., 2020) and a study of insectivorous bat diets which included coleopteran fragments (Sin *et al*., 2020). We recorded 22 morphospecies of aquatic beetles, a figure similar to that of Freitag *et al*. (2018), although lower than numbers documented by some studies elsewhere in Southeast Asia (e.g., Freitag & Zettel, 2013). As a whole however, few studies have investigated the diversity, distribution and environmental associations of aquatic insects in Cambodia to date.

We found that some beetle families were much more broadly distributed (e.g., Scirtidae & Elmidae) than others (e.g., Gyrinidae & Chrysomelidae). This may be explained by their differing life histories. For instance, Scirtidae larvae mostly occur in water, whereas the adults occur in terrestrial ecosystems (similar to Odonata, Ephemeroptera, Plecoptera & Trichoptera). In contrast, both life stages (larvae & adults) of Dytiscidae, Hydrophilidae and Elmidae can usually be found in water (Jäch & Balke, 2008). Since Coleoptera is a large order, ecological adaptations within the group are more diverse compared to other orders (Balke *et al*., 2014).

The habitat associations of aquatic beetles vary between families (Slipinski *et al*., 2011). Scirtidae, the most common family in our study, was negatively associated with the presence of agricultural land but positively related to forest cover and shaded areas, particularly at upstream sites (S01–S03). Given that Scirtidae occur on all continents and includes ≈1,400 species (Slipinski *et al*., 2011), its abundance at Chambok is unsurprising. Scirtidae larvae typically live in water and sometimes in wet soil whereas the adults emerge to surrounding forests (Lawrence, 2005; Yoshitomi & Sato, 2005). Streams characterised by low flow velocities and high proportions of aquatic plants have also been found to harbour high abundances of the group (Gooderham & Tsyrlin, 2002; Lawrence & Ślipiński, 2011).

We found that Hydrophilidae was positively correlated to high proportions of agricultural land use. Members of this family can be found in marsh vegetation-associated wetlands, side puddles of lakes and decaying organic plant matters in irrigated rice paddy fields (Freitag, 2015; Marsh *et al*., 2016). In contrast, Elmidae occurred at high abundance in wider stream areas characterised by deeper water and a greater quantity of large stones on the substrate. Members of this family are known as riffle beetles and typically occur in the rocky habitat of a fast-flowing streams and rivers. The larvae of the family also prefer microhabitats among stones and a high percentage of moss or plant roots (Elliott, 2008).

We also found that Dytiscidae, commonly referred as predaceous dying beetles, was positively associated with substrates characterised by accumulations of small stones. This was exemplified by our downstream sampling site (S10) which had a slow current. Previous reports indicate that this family occurs in most types of freshwater habitats including lakes, rivers, small stagnant water bodies, groundwater, streams and seepages (Jäch & Balke, 2008; Yee, 2014).

In conclusion, our study suggests that aquatic beetle communities in Chambok Commune are dominated by members of the Scirtidae and to a much lesser extent, the Elmidae and Dryopidae, whereas the presence of families such as Gyrinidae and Chrysomelidae is comparatively marginal. They also suggest that the occurrence of larvae and adults of these families varies in response to different types of stream microhabitats which consequently support different species assemblages. While further sampling is desirable to confirm and extend these findings, our study nonetheless provides a useful baseline for future research efforts on aquatic beetles in Cambodia.

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Annex 1 Environmental characteristics of sampling sites

Variable	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
Elevation (m)	425	394	391	2 93	241	197	150	133	117	116
Water Temperature (°C)	23.71	23.77	23.70	23.69	24.07	24.23	24.42	24.42	24.24	24.19
Water depth (m)	0.37	0.47	0.24	0.44	0.42	0.40	0.45	0.46	0.29	0.24
Stream width (m)	6.60	5.60	10.70	20.50	9.80	13.50	8.80	6.20	6.60	8.00
Erosion level	1	2	1	1	1	0	2	2	2	2
Large stones (>250 mm) (%)	60	50	45	65	45	48	55	65	55	35
Medium stones (64–250 mm) (%)	15	25	15	25	15	20	20	10	25	25
Small stones (2–64 mm) (%)	10	15	10	5	20	15	7	10	7	25
Sand (<2mm) (%)	10	8	10	3	10	10	5	10	3	10
Silt / clay (%)	5	2	20	2	10	7	13	5	10	5
Forest (%)	70	100	80	80	60	70	60	20	20	10
Agriculture (%)	0	0	0	0	0	0	0	45	50	40
Residential areas (%)	0	0	0	0	0	0	0	0	0	0
Orchards (%)	0	0	0	0	0	0	0	0	0	0
Bamboo (%)	30	0	20	20	40	30	40	35	30	50
Shade / canopy (%)	75	80	95	75	95	70	92	40	40	60