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A new association between Harpellales, insect-gut inhabiting fungi, and Chironomidae in Japan with an updated list of Harpellales documented from Chironomidae

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Abstract

Harpellales (Zoopagomycotina) is a fungal order of which species inhabit the intestine of aquatic arthropods by attaching their thalli to the host's gut lining. Harpellales consists of 270 species with over half found in Chironomidae larvae. The aim of this research is to document a species, *S. pedifer*, new to Japan with a chironomid host association and to list all Chironomidae-commensal species of Harpellales with host and country information.

Introduction

Harpellales is an order of Zoopagomycotina. In the old taxonomy, it belonged to Trichomycetes in Zygomycota. Species in Harpellales inhabit the digestive tracts of aquatic arthropods: mainly juveniles of Ephemeroptera, Plecoptera and Diptera, a few species in larvae of Trichoptera, and one species from both Coleoptera and Isopoda (Lichtwardt et al. 2001).

Infection begins with the ingestion of spores. After the germination in the gut, young thalli attach to the gut lumen with holdfasts. Thalli develop and produce spores in the intestine. Spores released from the anus to the environment will be the inocula for the new infection. Each genus of Harpellales has site specificity to the midgut (peritrophic membrane) or hindgut cuticle and young thalli attach to the defined site on the lumen. Species in this group live as commensals in the host gut and show no pathogenicity to the host insects. Only one exception, *Smittium morbosum*, has been reported to have a pathogenicity to kill the host mosquito larvae (Sweeney 1981).

Many species of Harpellales have been described from Diptera larvae. Chironomidae is an important group among host insects, hosting many species of Harpellales. Most studies of Harpellales focus on taxonomy and phylogeny, while research on ecological aspects is limited and mainly studied in species associated with Simuliidae (Taylor et al. 1996, Beard and Adler 2002, Nelder et al. 2010, Suyama et al. 2018), and in infection experiments in Culicidae (Williams and Lichtwardt 1972, Sato and Aoki 1989, Vojvodic and McCreadie 2007). Though Harpellales is an organism associated with host insects, many species have been described without exact host identification. The aim of this paper is to document a new association between Harpellales and Chironomidae in Japan and to provide an updated global list of Chironomidae-associated species of Harpellales and their chironomid hosts.

Materials and Methods

Chironomidae larvae (Orthocladinae sp.) were collected in a thin film of water flow beside Yoro River, close to Awamata Fall (Yoro Fall), Chiba prefecture, at 150m above sea level, in Feb. 2014 and Mar. 2015. Host insects were dissected to observe Harpellales. The larvae were individually placed on glass slides and the head and end of their abdomens were removed with a razor blade. The digestive tracts were pinched and removed from the abdomen with sharp forceps. Gut content was rinsed in a drop of water on the glass slide. The cleaned gut tube was then observed in water mounted on a new glass slide. Specimens were examined with a Nikon Optiphoto II microscope with a differential interference contrast apparatus or a Nikon Eclipse 2 microscope with a phase contrast apparatus (Nikon, Tokyo, Japan). Observations were conducted with ×10, ×20, ×40, and ×100 oil objective lenses in both microscopes. Photo images were taken with the Nikon DS-Fi 3 digital photo system (Nikon). After the observation, the mounting water was replaced by lactophenol, and the coverslips were sealed with nail polish. Vouchers are kept in the collection of the insect management laboratory, Department of forest entomology, Forestry and Forest Products Research Institute. Specimen ID: 140224-1. 140225-1 and 4, 150211-1, and 3.

Research achievements accumulated to date on Harpellales are presented in a website (Lichtwardt et al. 2001, revised in 2019). In this article, the information on this website is combined with the findings by Kim (2019), Sato (2020), and Sato (2022), and the fungal species recorded from Chironomidae are presented.

Results

New association between Harpellales and Chironomidae in Japan

A *Stachylina* sp. was recognized as new to Japan. The genus *Stachylina* attaches to the peritrophic membrane of host insects (midgut-specific genus), having a single simple (non-branched) thallus with two to dozens of spores. The morphological characters of the specimens fit the diagnosis of genus *Stachylina*. The *Stachylina* species has a thallus 56–107.5 µm in length and 6.3–9.3 µm in width, and produces two to eight long ellipsoid spores, 19.9–30.0 × 6.5–11.2 µm (25.5 ± 2.7 × 8.4 ± 1.3, average ± SD, N=45), without a collar and with an appendage. The basal part of the thallus penetrates through the peritrophic membrane by footlike cell apex (Fig. 3).

Species number detected from Diptera among Harpellales

Harpellales hold 270 species. Diptera hosts the highest number of Harpellales species of all insect hosts (188 Harpellales species in total). Chironomidae larvae are hosts to just over half of the known species of Harpellales (139 species from 10 genera, Figs 1, 2). The two largest genera, *Smittium* and *Stachylina*, each have 85 and 39 species respectively documented from Chironomidae larvae (Table 1, 2). Other minor genera with chironomid hosts constitute the remaining 15 species in 8 genera (Table 3).

Smittium aggregatum (Fig. 4) is shown, recently reported in Japan by Sato (2022), as an example of the genus *Smittium* which is the most common genus in association with Chironomidae. Species in *Smittium* attach to the hindgut cuticle specifically, having branched hyphae producing several spores at the distal end of the hyphae.

Distribution

The distribution of Chironomidae-associated Harpellales species is included in Tables 1-3. There are 28 countries with records, the most species are recorded from the United States and Canada, followed by France and Spain. In Japan, 10 species in three genera have been reported as associated with Chironomidae (*Stachylina grandispora*, *St. longa*, *St. magna*, *St. nana*, *St. penetralis*, *St. prolifica*, *St. subgrandis*, *Smittium aggregatum*, *Sm. elongatum*, *Trichozygospora chironomidarum*) (Lichtwardt et al. 1987, Sato 2020, 2022). *Smittium simulii* has been recorded form both Simuliidae and Chironomidae in Japan (Lichtwardt et al. 1987).

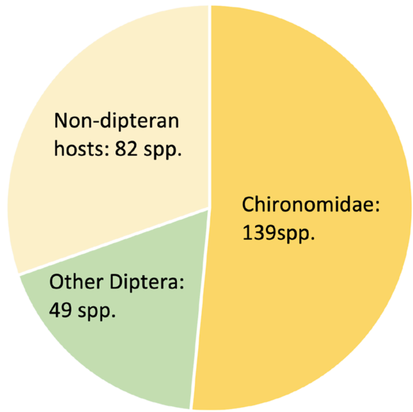

Figure 1. Total number of species in Harpellales described from Chironomidae and other hosts (N=270 species.)

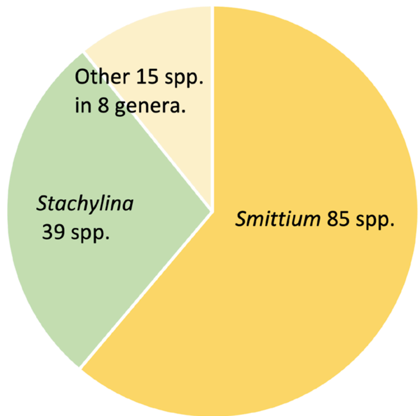

Figure 2. The species number in the two major genera of Harpellales described from Chironomidae (N=139 species)

Host identification and correspondence between fungi and hosts

At present, 23 species and 38 genera of Chironomidae have been identified as hosts of Harpellales. The most frequent host genera are *Cricotopus* (17 species in 4 genera of Harpellales), *Chironomus* (14 spp. in 3 genera), *Diamesa* (9 spp. in 3 genera), *Orthocladius* (8 spp. in 2 genera), *Polypedilum* (7 spp. in 2 genera), and *Tanytarsus* (6 spp. in 3 genera).

Of the 139 Chironomidae-commensal species, 69 species (about half) have hosts identified to genus or species, and 57 species (41 %) to family. *Smittium* has seven hosts identified to species and 35 to genus. *Stachylina* has seven hosts identified to species and 10 to genus (Table 4).

In the genus *Smittium*, 23 species are known from

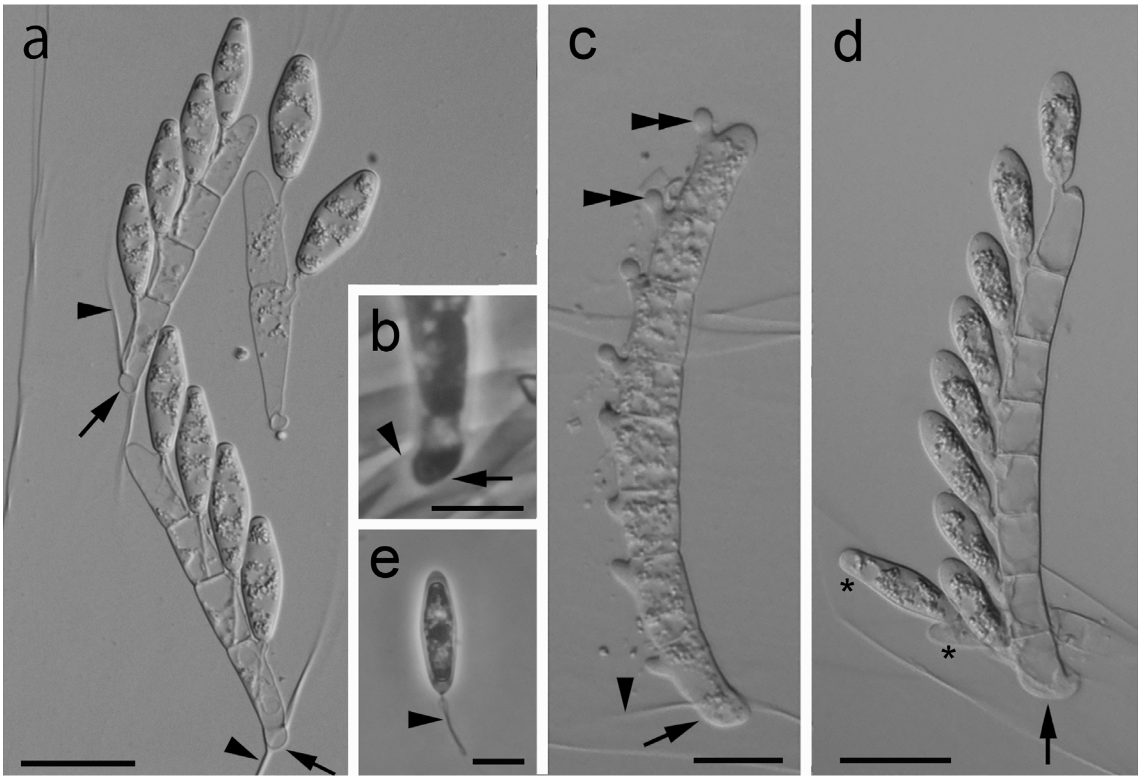

Figure 3. *Stachylina pedifer*. a. Mature thalli in the peritrophic membrane. Arrows: foot-like shaped basal part penetrating the peritrophic membrane. Arrowheads: folding peritrophic membrane. Also, the same symbols mean the same meaning in figs b, c, and d. b. Higher magnification of the basal part of a thallus. c. Young thallus producing spores. Double arrowheads: spore initial. d. Almost mature thallus with eight spores. *: Spores of adjacent thallus. e. Detached spore. Arrowhead: appendage. Scales. a: 20 µm. b–e: 10µm. Specimen ID. a and b: 140224-1. e: 140225-4. Photos c and d are taken from temporary slides. a, c and d: water-mounted. b and e: Lactophenol-mounted.

multiple chironomid taxa. In particular, *Smittium phytotelmatum* has been recorded from more than 5 taxa (Table 1). In the genus *Stachylina*, six species have been recorded from multiple host species. For example, *St. grandispora* is known from seven host taxa (Table 2). *Furculomyces boomerangus* has been recorded from four host taxa (Table 3).

There have been several records of Chironomidae species as a host of multiple species of Harpellales. The first case is the simultaneous infection of two Harpellales species to one individual larva of Chironomidae: *Boreoheptagyia lurida* was infected with *Stachylina pedifer*, and *Smittium dimorphum* (Lichtwardt and Williams, 1983), *Chironomus oppositus* group, and *Tanytarsus* near *inextentus*, both host species also with *Stachylina grandispora*, and *Furculomyces boomerangus* (Lichtwardt and Williams, 1990), and Tanitarsini sp. infected with *St. subgrandis*, and *Sm. aggregatum* (Sato 2022). The second case is without the description of simultaneous infection from the same collection. *Chironomus plumosus* complex was recorded as host of *St. euthena* and *Sm. typhellum* (Manier and Coste 1971), and *Tanytarsus* near *inextentus* was recorded as a host of *Sm. paludis*, and *F. boomerangus* (Lichtwardt & Williams 1990). The reported fungal species were derived from the same collection in the above two hosts' cases. However, the papers do not reveal if there were simultaneous infections or not.

The third case is from independent collections: *Brillia* cf. *longifurca*, host for *St. lentica* and *St. acutibasilaris* (White and Lichtwardt 2004); *Chironomus oppositus*, host for *F. boomerangus* and *F. westraliensis* (Lichtwardt and Williams 1990, 1992); and *Psectrotanypus varius*, host for *St. euthena* and *Sm. bisporum* (Manier and Coste 1971).

Discussion

The *Stachylina* species, derived from Orthocladinae sp., has a foot-like basal part that penetrates the peritrophic membrane. This morphology is the most prominent characteristic of *Stachylina pedifer*. Judging other morphological characteristics (thallus length, spore sizes, with an appendage, and without a collar) showed no remarkable differences from the original description of *St. pedifer* by Lichtwardt and Williams (1983). This species is therefore identified as *Stachylina pedifer* and is

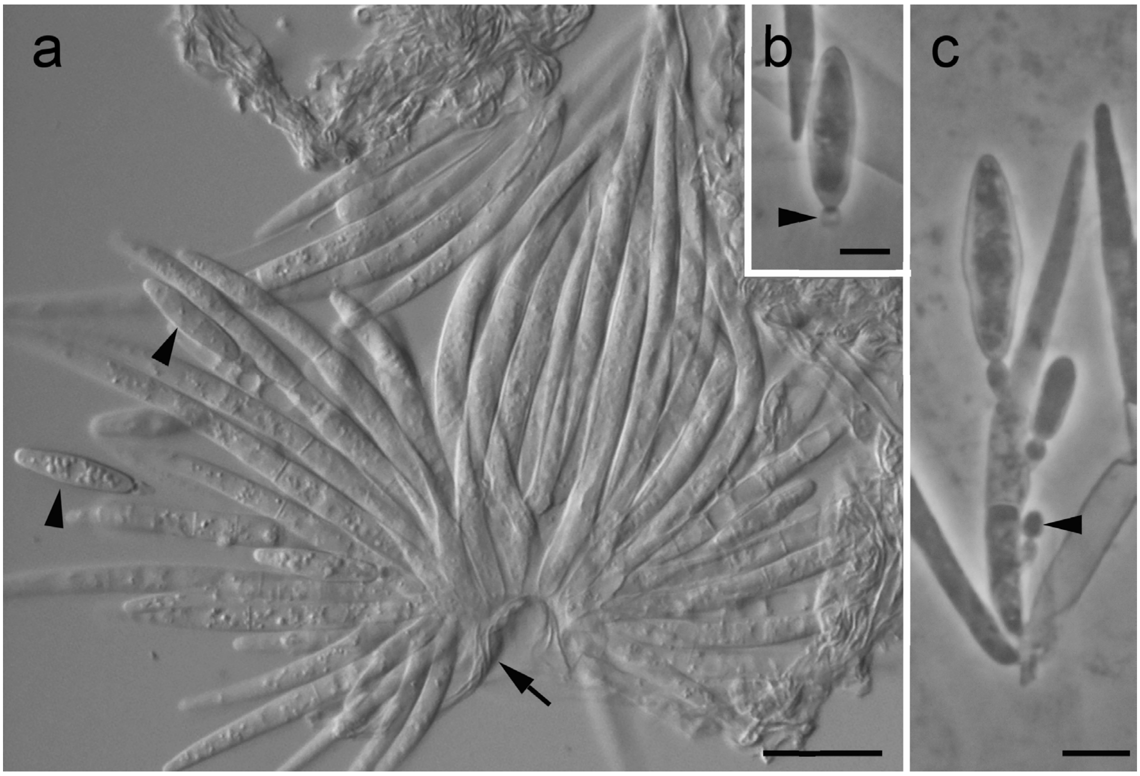

Figure 4. *Smittium aggregatum*. a. Thallus in the hindgut. Arrow: Hindgut cuticle (folded when dissected). Arrowheads: spores. Hyphal aggregation at the basal area of the thallus is the feature of this species. b. Detached spore. Arrowhead: Collar. Appendage is not reported in this species. c. Spore production. Arrowhead: The youngest spore. Scales. a: 20 µm. b–c: 5µm. Specimen ID. a: TNS-F-89237. b and c: TNS-F-89234. National Museum of Nature and Science, Tokyo. Host: larvae of Tanytarsini collected at Enzan Takahashi, Yamanashi, 1300m above sea level. a: water-mounted. b and c: Lactophenol-mounted.

the first record of this species in Japan. The known host insects of this fungus are *Boreoheptagyia lurida* (Diamesinae) (Lichtwardt and Williams 1983, White and Lichtwardt 2004) and *Eukiefferiella* sp. (Orthocladiinae) (Valle 2007).

Stachylina grandispora, *Smittium culicis* and *Sm. simulii* have been recorded from more than 10 countries (Tables 1 and 2). This distribution is of biogeographical interest. Furthermore, since some species of chironomids are found in severe environments, it is of interest to know whether Harpellales can adapt to such extreme conditions. For example, *Smittium incrassatum* has been collected in Greenland (Kobayasi et al. 1971).

The same fungal species would be detected from different host species in different countries. It is important to consider the environmental commonalities between the collection sites. *Trichozygospora chironomidarum* was reported in Japan from larvae of *Orthocladius* sp. (Sato 2020). The larvae were collected from the algal mass attached to the surface of rocks under fast-running water. The habitat was similar to the place of the original description (Lichtwardt 1972). The fungus was originally described from the United States infecting *Orthocladius* spp. and *Cricotopus* sp. larvae (Lichtwardt 1972). Subsequently, the species was recorded in Sweden from *Orthocladius* (*Euorthocladius*) and *Diamesa valkanovi* (Moss & Lichtwardt 1977, Lichtwardt et al. 2001), Switzerland, and England (no host information in these two countries) (Lichtwardt et al. 2001). The British record, however, needs to be re-examined (shortage of specimens). In the United States, this fungus was collected in the summer at about 2000 m above sea level. The Swiss site is also at about 2000 m above sea level, and the Swedish site is within the Arctic Circle. The Japanese collection site is the southernmost and is at 190 m above sea level. The specimens were collected in winter, however, and the habitat conditions in which this species is recorded may indicate that it is cold adapted.

The simultaneous association of multiple Harpellales species is also known in blackflies (Simuliidae). Especially, multiple fungal cohabitations have been known in the hindgut. However, cohabitation in the hindgut has not been reported in

Table 1. Host insects of *Smittium* based on Lichtwardt et al. (2001), Strongman and Xu (2006), Strongman et al. (2010), Wang et al. (2010), Strongman and Wang (2015), Kim (2019), Strongman and White (2019), and Sato (2022). All members except one of *Smittium* species are recorded from dipteran larvae (mainly Chironomidae). The exception: *S. foliocistum* from Trichoptera. *Hosts other than Chironomidae. # Possible distribution (Lichtwardt et al. 2001). AM: Armenia. AR: Argentina. AU: Australia. BR: Brazil. CA: Canada. CH: Switzerland. CL: Chile. CN: China. CR: Costa Rica. DK: Denmark (Greenland). DO: Dominican Republic. EN: England. ES: Spain. FR: France (including Crozet isl.) . JP: Japan. KR: Korea. MX: Mexico. NO: Norway. NZ: New Zealand. RU: Russia. SE: Sweden. TN: Tunisia. US: United States.

	Species	**Host**	**Distribution**
1	*Smittium aciculare*	Chironomidae, Simuliidae*	AU, MX
2	*Smittium acutum*	Chironomidae	US
3	*Smittium adaiosporum*	Chironomidae	CA
4	*Smittium aggregatum*	Tanitarsini	CA, JP
5	*Smittium alpinum*	*Diamesa* spp., other Chironomidae	CA, CH, FR, SE, US
6	*Smittium ampliboja*	Chironomidae, Simuliidae*	CA
7	*Smittium angustum*	*Cricotopus* sp.	AU
8	*Smittium annulatum*	Simuliidae*	CR
9	*Smittium arcticum*	Chironomidae	US
10	*Smittium arvernense*	*Smittia* sp.	FR
11	*Smittium basiramosum*	*Polypedilum* sp.	AR
12	*Smittium biforme*	*Diamesa aberrata*, possibly *D. bertrami*	NO
13	*Smittium bisporum*	*Psectrotanypus varius*	FR, MX#
14	*Smittium brasiliense*	*Paratendipes* sp., *Polypedilum* sp., Simuliidae*	BR, MX
15	*Smittium brevisporum*	*Cricotopus* spp. and other Chironomidae	CA, ES
16	*Smittium bulbosporophorum*	Diamesinae, Orthocladiinae	ES
17	*Smittium bullatum*	*Nanocladius forsythi*	NZ
18	*Smittium caribense*	Chironomidae	DO
19	*Smittium caudatum*	*Orthocladius* (*Orthocladius*) sp., *Cricotopus* sp., *Hydrobaenus* sp.	US
20	*Smittium cellaspora*	*Sympotthastia* sp. and possibly other Chironomidae	AR, US
21	*Smittium chinliense*	Tipulidae*	CN
22	*Smittium chironomi*	Orthocladiinae	EN#, FR
23	*Smittium colboi*	Chironomidae	CA
24	*Smittium coloradense*	*Orthocladius* (*Orthocladius*) sp., *Eukiefferiella* sp., *Cricotopus* sp., Simuliidae[3]	NO, US
25	*Smittium commune*	*Microtendipes* sp., *Cricotopus* (*Cricotopus*) sp., *Orthocladius* (*Orthocladius*) sp., *Phaenopsectra* sp.	US
26	*Smittium compactum*	Chironomidae	AU
27	*Smittium cryptancora*	Chironomidae	CA
28	*Smittium culicis*	Chironomidae, Culicidae[3], Simuliidae[3], Psychodidae[3],Stratiomyiidae[3], Thaumaleidae[3]	AR, AU, CA, CN, ES, FR, NO, NZ, TN, US
29	*Smittium culicisoides*	Chironomidae, Simuliidae[3]	AU#,CR, FR,
30	*Smittium cylindrosporum*	*Paraheptagyia* sp., *Paraheptagyia cinerascens*, *Cricotopus* sp.	AR, CL
31	*Smittium delicatum*	*Chironomus alternans*, *Cladopelma* sp., *Phaenopsectra* sp.	AU, US
32	*Smittium dimorphum*	*Boreoheptagyia lurida*	US
33	*Smittium dipterorum*	Chironomidae, Simuliidae*	CR, DO, MX
34	*Smittium ditrichosporum*	Chironomidae	CA
35	*Smittium elongatum*	*Diamesa* near *nivoriunda*, possibly *Cricotopus* sp.	AU, JP, US

36	*Smittium esteparum*	*Cricotopus* sp., *Eukiefferiella* sp.	AR
37	*Smittium fasciculatum*	Chironomus sp.	CR
38	*Smittium fastigatum*	Chironomidae	AU
39	*Smittium fecundum*	*Psectrocladius* sp.	ES, US
40	*Smittium foliocistum*	Trichoptera*	CA
41	*Smittium fruticosum*	*Cricotopus* sp.	AU
42	*Smittium georgense*	Chironomidae	CA
43	*Smittium gigasporus*	*Pagastia* sp.	US
44	*Smittium gracillis*	*Chironomus* sp. Diamesinae	CA, ES
45	*Smittium gravimetallum*	*Dicrotendipes fumidus*	US
46	*Smittium gronthidium*	Chironomidae, Simuliidae*	CA
47	*Smittium guttisporum*	Chironomidae	CA
48	*Smittium hecatei*	*Cricotopus* spp. and Diamesini	CN, ES
49	*Smittium heterosporum*	*Sympotthastia* spp., *Potthastia* spp. (Diamesinae), *Cricotopus bicintus* (Orthocladiinae)	ES
50	*Smittium imitatum*	Simuliidae*	AR, CL
51	*Smittium incrassatum*	Chironomidae	DK
52	*Smittium inexpectans*	Orthocladiinae	ES
53	*Smittium insolitum*	Chironomidae	CA
54	*Smittium insulare*	Chironomidae, Culicidae*	CA
55	*Smittium kansense*	*Hydrobaenus* sp., *Orthocladius* (*Orthocladius*) sp.	US
56	*Smittium lentaquaticum*	*Chironomus* sp.	US
57	*Smittium longisporum*	*Cricotopus* sp. and other Chironomidae	SE, US
58	*Smittium macrosporum*	Chironomidae	US
59	*Smittium magnosporum*	Thaumaleidae*	AU
60	*Smittium manierei*	Chironomidae	FR
61	*Smittium megazygosporum*	*Syncricotopus rufiventris*	FR, KR
62	*Smittium microsporum*	*Tanytarsus* sp., possibly *Cricotopus* sp.	AU, MX
63	*Smittium minutisporum*	Culicidae*	CN, US
64	*Smittium morbosum*	Culicidae*	AM, AR, AU, IT, JP, RU
65	*Smittium mucronatum*	*Psectrocladius sordidellus*, *Psectrocladius* sp. *Psectrocladius limbellatus*	CA, FR, NO, US
66	*Smittium naiadis*	Chironomidae	CN
67	*Smittium nodifixum*	Chironomidae	CN
68	*Smittium orthocladii*	*Orthocladius* spp., *Diamesa* sp., and other lotic Chironomidae	FR, US
69	*Smittium ouselii*	*Eukiefferiella* sp.	US
70	*Smittium paludis*	*Tanytarsus* near *inextentus*	AU
71	*Smittium papillum*	Chironomidae	CA
72	*Smittium parvum*	*Cricotopus* sp.	CR
73	*Smittium pavocaudatum*	Chironomidae	CA
74	*Smittium peculiare*	Chironomidae	CA
75	*Smittium pennelli*	Simuliidae*	US
76	*Smittium perforatum*	*Orthocladius* (*Euorthocladius*) sp. *Diamesa* sp.	US
77	*Smittium petilum*	Chironomidae	CA
78	*Smittium phytotelmatum*	*Chironomus* sp., *Polypedilum* spp., *Tanytarsus* sp., and other Chironomini and Tanitarsini	AR, CR

79	*Smittium precipitiorum*	*Orthocladius fuscimanus*, *Diamesa* sp.	NO
80	*Smittium prostratum*	Orthocladiinae	ES
81	*Smittium pseudodimorphum*	Diamesinae	ES
82	*Smittium pusillum*	*Procladius* sp.	FR
83	*Smittium radiculans*	Simuliidae*	CA
84	*Smittium rarum*	Chironomidae	NZ
85	*Smittium rupestre*	Orthocladiinae	AU, CN
86	*Smittium scrobense*	Chironomidae	CA
87	*Smittium shaanxiense*	Chironomidae	CN
88	*Smittium simulatum*	Tipulidae*	CL
89	*Smittium simulii*	Chironomidae, Simuliidae*, Culicidae*, Tipulidae*	AU, CA, CN, EN, ES, FR, JP, KR, MX, NO, NZ, SE, US
90	*Smittium sparsum*	Chironomidae	CA
91	*Smittium tipulidarum*	Tipulidae*	US
92	*Smittium tronadorium*	*Paraheptagyia* sp., *Cricotopus* sp.	AM#, AR, MX
93	*Smittium tynense*	Chironomidae	CA
94	*Smittium typhellum*	*Chironomus* sp. (*plumosus* complex)	FR
95	*Smittium urbanum*	Chironomidae	AR
96	*Smittium verticillatum*	Chironomidae	CA

Table 2. World records of *Stachylina* spp. based on Lichtwardt et al. (1987), Lichtwaradt et al. (2001), Misra and Tiwari (2002), Hapsari et al. (2009), Strongman et al. (2010), Wang et al. (2010), Strongman and Wang (2015), Sato (2020). All *Stachylina* species have been recorded from larvae of Diptera (mainly Chironomidae). *Hosts other than Chironomidae. (+) New record from Japan in this study. # Possible misidentification. AR: Argentina. AU: Australia. BR: Brazil. CA: Canada. CH: Switzerland. CL: Chile. CN: China. CR: Costa Rica. DO: Dominican Republic. EN: England. ES: Spain. FR: France (including Crozet Isl.). IN: India. JP: Japan. MX: Mexico. NO: Norway. NZ: New Zealand. PT: Portugal. SE: Sweden. TH: Thailand. US: United States

	Species	Host identification	Records
1	*Stachylina abundans*	Chironomidae	CA
2	*Stachylina acutibasilaris*	*Brillia* cf. *longifurca*	NO
3	*Stachylina brevicellaris*	Chironomidae	CA
4	*Stachylina ceratopogo-nidarum*	Ceratopogonidae*	CL
5	*Stachylina chironomidarum*	Chironomidae	IN, US
6	*Stachylina dolichospora*	*Riethia* sp.	AU
7	*Stachylina euthena*	*Chironomus plumosus* complex, *Psectrotanypus varius*	ES, FR
8	*Stachylina extensiva*	Chironomidae	CA
9	*Stachylina forantipes*	Chironomidae	CA
10	*Stachylina grandispora*	*Chironomus hawaiensis*, *C. zealandicus*, *Chironomus* spp., *Cricotopus*, *Polypedilum*, *Paratendipes*, *Tanytarsus*	AR, CA, DO, EN, ES, IN, JP, MX, AN, SE, US
11	*Stachylina gravicaudata*	*Paralauterborniella* sp.	CN, US
12	*Stachylina infrequens*	Chironomidae	AU
13	*Stachylina jujuyensis*	Chironomidae	AR
14	*Stachylina lentica*	*Chironomus* sp., *Brillia* cf. *longifurca*, *Polypedilum* sp. *Metriocnemus eryngiotelmatus*	AR, NO
15	*Stachylina litoralis*	*Telmatogeton japonicus*, Simuliidae*	CA, FR
16	*Stachylina longa*	*Tanytarsus* sp.	FR, JP

17	*Stachylina lotica*	Psychodidae*	AR, US
18	*Stachylina macrospora*	*Diamesa* sp., *Syndiamesa macronyx*	FR, IN, US#
19	*Stachylina magna*	Chironomidae	JP, US
20	*Stachylina manicata*	*Polypedilum* sp. and/or *Pseudochironomus* sp.	US
21	*Stachylina minima*	Orthocladiinae	AR, NZ
22	*Stachylina minuta*	Chironomidae	FR
23	*Stachylina nana*	Chironomidae	CA, CN, R, JP, TH, US
24	*Stachylina paludosa*	*Chironomus* sp., *Polypedilum* sp.	CR
25	*Stachylina paucispora*	*Cricotopus* sp.	BR, MX
26	*Stachylina pedifer* (+)	*Boreoheptagyia lurida*, *Eukiefferiella* sp.	CA, CN, ES, JP, NO, US
27	*Stachylina penetralis*	*Diamesa* and other Chironomidae	AR, CA, CH, CN, ES, FR, JP
28	*Stachylina philoricola*	Blephariceridae*	JP
29	*Stachylina platensis*	Chironomidae	AR
30	*Stachylina pleurospora*	Chironomidae	ES, PT
31	*Stachylina prolifica*	*Chironomus* sp.	CN, ES, JP
32	*Stachylina queenslandiae*	Chironomidae	AU
33	*Stachylina robusta*	*Psectrocladius* sp.	ES, US
34	*Stachylina serpula*	Chironomidae	CA
35	*Stachylina somnisimilis*	Chironomidae	CA
36	*Stachylina stagnicola*	Chironomidae	CA
37	*Stachylina stenospora*	*Phaenopsectra* sp.	US
38	*Stachylina subgrandis*	Chironomidae, Tanitarsini sp.	CA, CN, JP
39	*Stachylina tanysoma*	Chironomidae	CA
40	*Stachylina thaumaleidarum*	Thaumaleidae*	AU
41	*Stachylina tianensis*	Chironomidae	CN
42	*Stachylina uranus*	Chironomidae	CA
43	*Stachylina zeppelin*	Chironomidae	CA

Table 3. Host insect of Harpellales associated with Chironomidae except the genera *Smittium* and *Stachylina* based on Lichtwardt et al. (2001), Strongman and Xu (2006), Strongman et al. (2010), Wang et al. (2010), Strongman and Wang (2015), Kim (2019), Strongman and White (2019) and Sato (2020). *Zancudomyces culisetae* has wide host range of insect groups other than Chironomidae. # Possible distribution because of specimen shortage (Lichtwardt et al. 2001). AR: Argentina. AU: Australia. BR: Brazil. CH: Switzerland. CN: China. DO: Dominican Republic. EN: England. FR: France (including Crozet isl.). JP: Japan. MX: Mexico. NZ: New Zealand. SE: Sweden. US: United States.

	Species	Host identification	Records
1	*Austrosmittium aussiorum*	near *Kiefferophyes*, *Paratrichocladius pluriserialis*	AU
2	*Austrosmittium biforme*	Orthocladiinae	AU
3	*Austrosmittium kiwiorum*	Orthocladiinae	NZ
4	*Austrosmittium lenticum*	*Corynoneura* sp.	AR
5	*Austrosmittium norinsulare*	Orthocladiinae	NZ
6	*Austrosmittium patagonicum*	*Parochlus* sp.	AR
7	*Dacryodiomyces oklahomensis*	*Cricotopus* sp.	US
8	*Furculomyces boomerangus*	*Aspectrotanypus maculosa*, *Chironomus oppositus*, *Procladius paludicola*, *Tanytarsus* near. *inextentus*	AU

9	*Furculomyces septentrionalis*	*Chironomus* sp.	US
10	*Furculomyces westraliensis*	*Chironomus oppositus* group	AU
11	*Klastostachys reflexa*	*Cryptochironomus* sp.	US
12	*Sinotrichium chironomidarum*	Chironomidae	CN
13	*Stachylinoides arctata*	*Thienemanniella* sp.	AR
14	*Trichozygospora chironomidarum*	*Cricotopus* sp., *Diamesa valkanovi*, Orthocladius sp.	CH, EN#, JP, SE, US
15	*Zancudomyces culisetae*[2]	Chironomidae, Culicidae, Simuliidae, Psychodidae, Ceratopogonidae, Ephemeroptera	US, JP, AU, NZ, BR, AR, FR, DO, MX

Table 4. Number of species in Harpellales at each identified chironomidae rank.

Taxa	species	genus	tribe	subfamily	family	total
Smittium	7	35	1	6	36	85
Stachylina	7	10	1	2	19	39
other	4	6	0	3	2	15
total	18	51	2	11	57	139

Chironomidae. Lichtwardt (1972) reported two or more fungal species associated with the hindgut of blackfly larvae among a decade of collections in the USA. In Japan, a fungal species in the midgut and two species in the hindgut have been reported (Sato 2013, Suyama et al. 2018).

Fixed point observations are effective to reveal host-associate correspondence. By repeating the collection effort of the same host species at a fixed point from 2003 to 2017, four species associated with *Simulium japonicum* have been recognized in Japan (Sato and Degawa 2003, Sato and Orihara 2013, Suyama et al. 2017, 2018). Moreover, the periodical collection at fixed points has revealed seasonal fluctuation in the prevalence of each associating Harpellales in one host species (Suyama et al. 2018), and also the difference of prevalence between sympatric other host species (Taylor et al. 1996, Beard and Adler 2002, Nelder et al. 2010). Researches using fixed point are expected for Chironomidae-associated Harpellales.

The large number of fungal species recorded from multiple hosts suggests that the host specificity of Harpellales in Chironomidae may not be strict. On the other hand, the host identification rank for 57 Harpellales species is still family level. At the same time, many species of Harpellales have been recorded from only one country where they were described. As new hosts are identified, and also as new ecological information is derived, more detailed discussions of the fungus-host relationship are allowed. The interrelationship between fungi and insects is expected to be elucidated through the accumulation of records from different countries and regions by entomologists and mycologists.

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