



Strongwellsea tigrinae and Strongwellsea acerosa (Entomophthorales: Entomophthoraceae), two new species infecting dipteran hosts from the genus Coenosia (Muscidae)



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ARTICLE INFO

Keywords:
Insect pathogenic fungus
Muscid flies
Diversity
Host specialization

ABSTRACT

Two new species from the genus *Strongwellsea* (Entomophthorales: Entomophthoraceae) are described: *Strongwellsea tigrinae* from adult *Coenosia tigrina* (Diptera: Muscidae) and *Strongwellsea acerosa* from adult *Coenosia testacea*. The descriptions are based on pathobiological, phenotypical and genotypical characters. Further, the circumscription of the genus *Strongwellsea* is emended. Our findings suggest that *Strongwellsea* harbors a high number of species, of which now only five have been described.

1. Introduction

Strongwellsea Batko & Weiser is a genus of fungi from Entomophthorales whose species are specialized as pathogens on adult Diptera. The genus belongs to the Entomophthoraceae subfamily Erynioideae (erynid or, more commonly, zoophthroid clade), whose several genera produce mononucleate, bitunicate conidia (Jensen et al., 1998; Gryganski et al., 2012, 2013; Humber, 2016). According to the findings of Batko and Weiser (1965), Humber (1975, 1976), Eilenberg and Michelsen (1999), Eilenberg et al. (2000, 2020) the *Strongwellsea* generic characters are the following: Vegetative growth is principally restricted to the abdomen, although some few hyphae can be found elsewhere in the body. The fungus forms a hymenium-lined ball creating a cavity in the abdomen and opening one or, more rarely, two large circular or oval holes in the ventral abdomen of a living host. Conidiophores are simple, each discharging one primary conidium towards the abdominal hole. Primary conidia are obovoid to cylindrical, mononucleate, bitunicate, and with a rounded papilla. Primary conidia can form two types of forcibly discharged secondary conidia, of either the ellipsoid or subglobose type (Eilenberg et al., 2020). Especially towards the end of the season, *Strongwellsea* in some infected flies develops orange, globose resting spores with a spiny episore filling the abdomen instead of conidia.

Strongwellsea infections have been documented in the northern hemisphere on adult calyprate Diptera belonging the families Anthomyiidae (Smith, 1927; Strong et al., 1960; Jones, 1970; Nair and McEwen, 1973,

Evlakova, 1974; Eilenberg and Michelsen, 1999; Klingen et al., 2000); Fanniidae (Humber, 1976; Eilenberg and Michelsen, 1999; Eilenberg and Jensen, 2018); Muscidae (Eilenberg and Michelsen, 1999; Eilenberg, 2002; Keller, 2007); Scathophagidae (Eilenberg and Jensen, 2018); Calliphoridae, and Sarcophagidae (Eilenberg and Michelsen, 1999). The only example from Diptera subsection Acalyptratae is from Tephritidae (Aoki and Shiga, 1988). Three species have been described: *Strongwellsea castrans* Batko and Weiser (1965) from host *Delia platura* Meigen (Anthomyiidae); *Strongwellsea magna* Humber (Humber, 1976) from host *Fannia canicularis* L. (Fanniidae); and *Strongwellsea pratensis* Keller from host *Coenosia albicornis* Meigen (Muscidae) (Keller, 2007). In all cases, the species description was primarily based on fungal morphology, especially the dimensions (shape, length, width and length/width ratio) of primary conidia.

Eilenberg and Michelsen (1999) documented a clear correlation between morphology of *Strongwellsea* primary conidia and infected host species in 14 dipteran host species from Muscidae, Anthomyiidae, Fanniidae, and Calliphoridae. The morphology of conidia from natural *Strongwellsea* infections in one host species was consistently different from *Strongwellsea* conidia from other host species. This was also the case when hosts belonged to the same family (Anthomyiidae or Muscidae), although *Strongwellsea magna* conidia from three closely related host species from the genus *Fannia* were similar, and *Strongwellsea castrans* sensu lato conidia from the host complex including *Delia florilega* Zetterstedt and *D. platura* were also similar. The results from a phylogenetic study by Eilenberg and Jensen (2018) analyzing ITS2 of

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Figs. 1–6. *Strongwellsea tigrinae*, infected hosts *Coenosia tigrina*. (1) Specimen with two abdominal holes. (2) Specimen with one abdominal hole. (3) Specimen with abdominal hole partly blocked. (4) Specimen filled with resting spores. (5) Specimen filled with resting spores. (6) Close up view of resting spores.

Strongwellsea from infections in 15 dipteran hosts correlated very well with the morphological studies. The authors found that each *Strongwellsea* genotype occurred in just one host species or a few closely related host species (species from genus *Fannia* and *D. florilega/D. platura*). Within Muscidae, *Strongwellsea* genotypes from each of four included host species clustered together well without including hosts from any other fly families but were, in turn, separated from each other with high bootstrap value support. This was even the case for *Strongwellsea* infections in the same host genus in Muscidae, *Strongwellsea* from host *Coenosia tigrina* (Fabricius) and from host *Coenosia testacea* (Robineau-Desvoidy) clustered separately. The latter species differed, however, from *S. magna* by just a few base pairs.

A combination of data about pathobiology (host species), fungal phenotypic characters such as conidial and resting spore morphologies, as well as ITS2 genotype led us to conclude that there is sufficient evidence to describe two new *Strongwellsea* species infecting *C. tigrina* and *C. testacea*, respectively, and to emend the generic characterization of *Strongwellsea* to reflect newly recognized variations within this group.

2. Materials and methods

2.1. Sampling and incubation

Coenosia tigrina was sampled October 4–28, 2019, at Vester Strand, Jægerspris, Denmark (55.8533045 deg N, 11.9647856 deg E). The area is mixed woodland, hedges and cropped fields. *Coenosia testacea* was sampled August 16 – September 2, 1998, at Sønderholm Allé, Amager, Denmark (55.6179207 deg N, 12.5966403 deg E). It is a residential area with houses and gardens. Flies were sampled with a sweep net. Living flies exhibiting abdominal holes and by that infections in the conidial stage were diagnosed *in situ* and incubated individually in 25 ml cups for collection of primary and secondary conidia to be kept dry on coverslips until examination (Eilenberg et al., 2020). Flies without symptoms were incubated one week at room temperature to obtain additional flies developing symptoms of conidial infections and to obtain flies dying from *Strongwellsea* infections in resting spore stage. Infected flies were identified to species level and afterwards kept individually in 70% alcohol if infected and discharging conidia, and kept dry if filled with resting spores.

2.2. Fungal morphology

A drop of lactic acid (without cotton blue or any other stain) was added to a glass slide, and coverslips with conidia were inverted onto it and sealed with nail polish. For nuclei, aceto-orcein was used to stain. In the case of resting spores, flies were partly dissected to obtain sufficient number of resting spores to stain and to examine. The morphological studies of primary conidia, secondary conidia, and resting spores were performed using an Olympus AX70 Provis microscope with differential interference contrast or phase contrast at 400 X magnification. Measurements were made only for those primary or secondary conidia situated laterally, with the axis parallel to the slide and with the whole spore outline in focus. Only resting spores without malformations due to pressure were measured. Length and width of up to 100 spores (primary conidia, secondary conidia, and resting spores), to a maximum of 20 spores per infected fly, were measured. Material from the holotypes and the isotypes were included in the microscopic measurements. The mean, 95% confidence interval and range are provided for length, width and length/width ratios.

2.3. DNA extraction and gene sequencing

Data on DNA extraction and ITS2 analysis were given by [Eilenberg and Jensen \(2018\)](#).

3. Results

Coenosia tigrina: nine infected specimens were collected on the type locality between October 4 and 19, 2019; all were females. Of these, seven had upon capture or developed after incubation one (in one case two) abdominal hole through which conidia were projected, while two infected flies died filled with resting spores and exhibited no abdominal hole. *Coenosia testacea*: 54 infected specimens were collected on type locality between August 16 and September 2, 1998; all except four were females. Of the infected flies, 52 had upon capture or developed after incubation one (in one case two) abdominal hole through which conidia were projected, while two infected flies died filled with resting spores and exhibited no abdominal hole.

Strongwellsea tigrinae Eilenberg & Humber, sp.nov. [Figs. 1–21](#)

Index Fungorum Registration # IF 556970

The infected host fly *Coenosia tigrina* (Fabricius) develops one or, rarely, two holes on the ventral abdomen ([Fig. 1, 2](#)). The holes are oval with the side being flattened towards the axis of the ventral abdomen. They gradually widen during the progression of the infection and may reach approx. 1 mm in the longest dimension. In the final stage of infection, the holes can be partly blocked by discharged conidia sticking to each other ([Fig. 3](#)). From a hymenium inside the hole, primary conidia are discharged from the living fly over several days and for a few hours after the host's death. Infected hosts may instead of developing a hole be filled with resting spores in abdomen. When the host is dead and filled with resting spores, the abdomen will gradually break apart ([Figs. 4, 5](#)). Resting spores are shiny dark orange with a coarsely spiny episore ([Figs. 6, 20](#)).

Primary conidia are obovoid, uninucleate, bitunicate with a lightly textured surface, and a broad base ([Fig. 7, 9](#)); mean length is $34.3 \pm 0.50 \mu\text{m}$ (range 27.1–40.4 μm); mean width is $17.0 \pm 0.11 \mu\text{m}$ (range 14.2–20.3 μm); length/width ratio is 2.02 ± 0.01 (range 1.76–2.26). Primary conidia can form two types of secondary conidia from lateral conidiophores; both types of conidia are actively discharged. Ellipsoid type secondary conidia form on conidiophores shorter or almost as long as the conidium itself ([Figs. 10, 11](#)); their surface is lightly textured, and the basal papilla is more or less sharply pointed ([Figs. 12, 13](#)); mean length is $31.4 \pm 0.59 \mu\text{m}$ (range 26.7–37.2 μm); mean width is $19.5 \pm 0.43 \mu\text{m}$ (range 16.0–22.6 μm); length/width ratio is 1.61 ± 0.03 (range 1.39–1.83). Subglobose type secondary conidia form on very short conidiophores ([Fig. 14](#)) with a smooth surface and flattened basal papilla ([Figs. 15, 16](#)); mean length is $19.2 \mu\text{m} \pm 0.56 \mu\text{m}$ (range 16.6–21.5 μm);

mean width is $15.4 \mu\text{m} \pm 0.46 \mu\text{m}$ (range 13.3–17.6 μm); length/width ratio is 1.25 ± 0.02 (range 1.17–1.34). Both types of secondary conidia may form a germ tube ([Figs. 17, 18](#)). Ellipsoid secondary conidia may form a tertiary conidium ([Fig. 19](#)). Resting spores are spherical to subspherical, a dark, shiny orange color, with a spiny episore ([Figs. 20–21](#)); mean length including episore is $44.8 \pm 1.49 \mu\text{m}$ (range 26.2–60.0 μm); mean width $43.6 \mu\text{m} \pm 1.47 \mu\text{m}$ (range 25.7–56.8 μm); length/width ratio 1.03 ± 0.01 (range 1.00–1.10).

HOLOTYPE: C-F-133663, The Natural History Museum, University of Copenhagen, Denmark. Infected host, *Coenosia tigrina*, sampled October 4, 2019, on type locality Vester Strand, Jægerspris, Denmark (55.8533045 deg N, 11.9647856 deg E), infected host stored in alcohol and conidia discharged onto slides.

ISOTYPES: The Natural History Museum, University of Copenhagen, Denmark. Infected hosts, *Coenosia tigrina*, sampled October 4–28, 2019, on type locality: C-F-133664 through C-F-133667, infected host and conidia stored in alcohol and conidia discharged onto slides; C-F-1336668 through C-F-133670, infected host with resting spores (stored dry).

Genbank accession numbers (for ITS2): MH703605, MH703606, MH703607 ([Eilenberg and Jensen, 2018](#)).

Etymology: The name refers to the host species.

Distribution and seasonality: Infected flies collected July to October at various localities in North Zealand, Denmark.

Similar species: *Strongwellsea pratensis* Keller infects *Coenosia albicornis*. The dimensions of primary conidia of this species ($25\text{--}30 \times 11\text{--}12 \mu\text{m}$) are, however, significantly different. There is, for example, no overlap between the widths of the primary conidia. The resting spores of *S. tigrinae*, being dark, shiny orange, are different from resting spores of *Strongwellsea castanea*, which are bright orange ([Humber, 1976, Eilenberg and Michelsen, 1999](#)).

Strongwellsea acerosa Eilenberg & Humber, sp.nov.

[Figs. 22–29](#)

Index Fungorum Registration # IF 556971

The infected host fly *Coenosia testacea* (Robineau-Desvoidy) develops one or, rarely, two holes on the ventral abdomen ([Fig. 22](#)). The holes are circular and approximately 0.5 mm wide. From a hymenium inside the hole, primary conidia are discharged over several days from the living fly and until a few hours after the death of the host. Some infected hosts do not develop a hole, instead the abdomen is filled with yellow resting spores ([Figs. 23, 23a](#)), the abdomen eventually breaks apart to expose these resting spores. When the host is dead and filled with resting spores, the abdomen will gradually break apart.

Primary conidia are acerose, with flattened basal papilla, smooth surface and tapering towards the apex ([Figs. 24, 25, 27](#)) and contain one nucleus almost filling the width of the conidium ([Fig. 26](#)); mean length is $29.0 \pm 0.50 \mu\text{m}$ (range 22.7–36.5 μm); mean width is $6.9 \pm 0.15 \mu\text{m}$ (range 5.4–9.1 μm); length/width ratio is $4.21 \pm 0.07 \mu\text{m}$ (range 3.45–5.13). No secondary conidia were observed. Instead, immediately after landing on a coverslip, primary conidia started to germinate from the apex and/or through the papilla ([Figs. 26, 28](#)). Resting spores are spherical to subspherical, yellow, with a spiny episore ([Fig. 29](#)); mean length (including episore) $38.1 \pm 1.01 \mu\text{m}$ (range 29.5–51.1 μm); mean width $37.0 \mu\text{m} \pm 1.05 \mu\text{m}$ (range 28.0–49.4 μm); length/width ratio 1.04 ± 0.03 (range 1.00–1.13).

HOLOTYPE: C-F-133671, The Natural History Museum, University of Copenhagen, Denmark. Infected host, *Coenosia testacea*, sampled August 16, 1998, on type locality Sønderholm Allé 33, Amager, Denmark (55.6179207 deg N, 12.5966403 deg E), infected host stored in alcohol and conidia discharged onto slides.

ISOTYPES: The Natural History Museum, University of Copenhagen, Denmark. Infected hosts, *Coenosia testacea*, sampled August 16–September 2, 1998, on type locality: C-F-133672 through C-F-133680, infected host stored in alcohol and conidia onto slides; C-F-133681 and C-F-133682, infected host with resting spores (stored dry).

GenBank accession number (for ITS2): MH703618 ([Eilenberg and Jensen, 2018](#)).

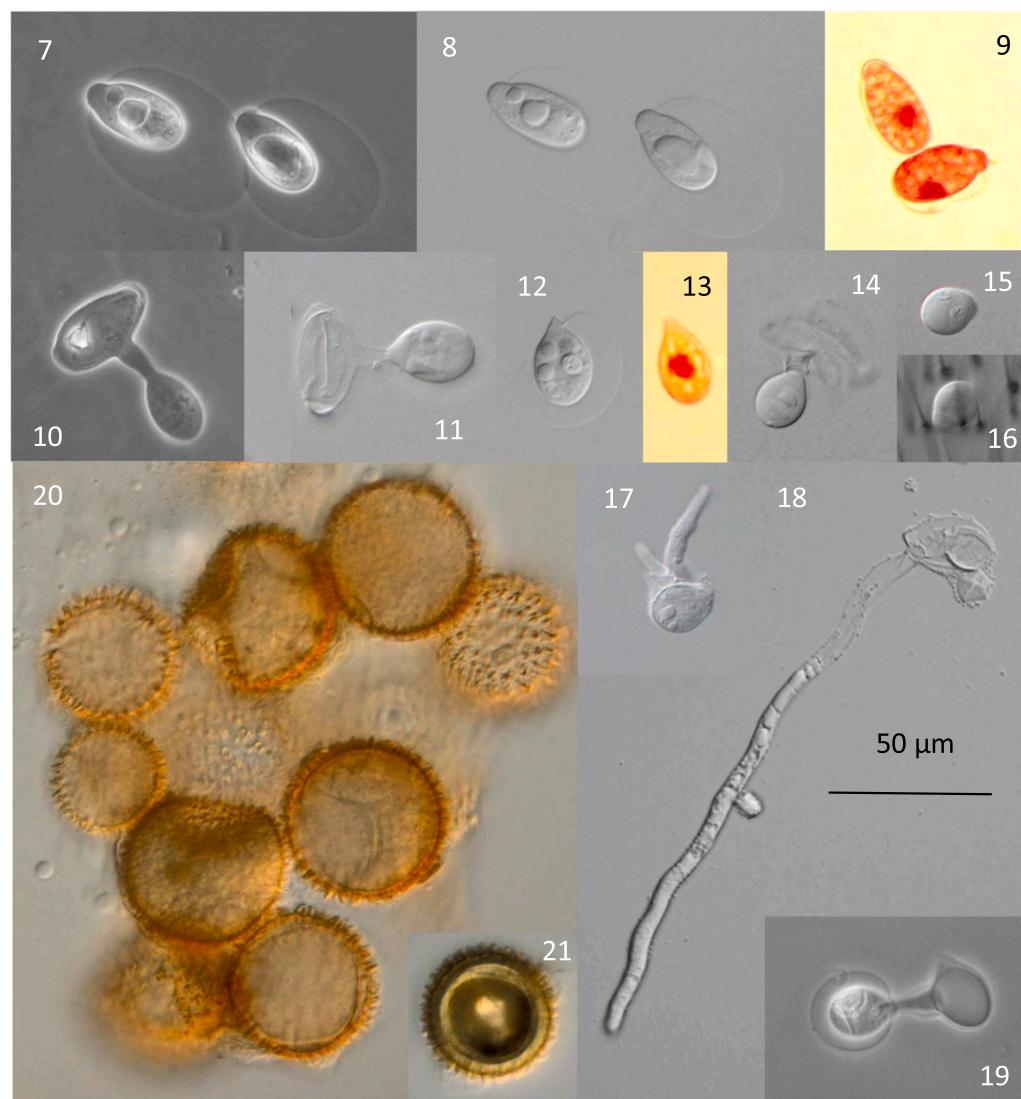


Fig. 7–21. *Strongwellsea tigrinae* spores. (7) Primary conidia. (8) Primary conidia. (9) Primary conidia, nuclei staining. (10) Primary conidium forming an ellipsoid type secondary conidium. (11) Primary conidium forming an ellipsoid type secondary conidium. (12) Ellipsoid type secondary conidium. The base is sharply pointed. (13) Ellipsoid type secondary conidium, nuclei staining. (14) Primary conidium forming a subglobose type secondary conidium. (15) Subglobose type secondary conidium. The basis is flattened. (16) Subglobose type secondary conidium on wing. (17) Germinating subglobose type secondary conidium. (18) Germinating ellipsoid type secondary conidium. (19) Ellipsoid type secondary conidium forming an ellipsoid type tertiary conidium. (20) Resting spores. (21) Fully mature resting spore. Light source: Interference Figs. 8, 11, 12, 14, 15, 16, 17, 18, 20, and 21; phase contrast Figs. 7, 10, and 19; transmitted light Figs. 9 and 13. Scale bar for all photos on Fig. 18.

Etymology: The name refers to the needle-shaped primary conidia.

Distribution and seasonality: Infected flies collected August 1998 on type locality near Copenhagen, so far not found elsewhere.

Similar species: There is no other species in the genus *Strongwellsea* with acerose primary conidia or a length/width ratio exceeding three. In addition, the distinctly yellow color of the resting spores is different from the orange color of all other described resting spores of *Strongwellsea* species (Humber, 1976, Eilenberg and Michelsen, 1999).

4. Emendation of the genus *Strongwellsea*

STRONGWELLSEA Batko & Weiser emend. Humber & Eilenberg. TYPE-*Strongwellsea castrans* Batko and Weiser (1965). J. Invert. Pathol. 7: 455–463. Parasitic in adult muscoid dipterans, restricted principally to the abdominal hemocoel, forming an expanding hollow ball in the hemocoel that attaches to the host's cuticle from the inside and opens a large nearly circular or oval hole in the ventral abdominal pleuron. Mycelium coenocytic, simple or infrequently branched, without a cell wall throughout most development in the host hemocoel, later forming simple or sparingly branched hyphal bodies but not circulating in the hemocoel and forming a hyphal mass that becomes moribund in the interior and then hollow; innermost hyphae acquiring cell walls before branching to form conidiophores; few hyphae occasionally grow into the thoracic hemocoel and enter the thoracic ganglion and proliferate

throughout the nervous tissues including the brain. Conidiophores simple, clavate, rising perpendicularly from layer of walled hyphae wrapping around the abdominal ball and forming a hymenium lining the innermost portion of the cavity in the host abdomen; hymenial cells adjacent to the hole short are swollen, prominently vacuolate, sterile, and covered by a hypertrophic extension of the host epicuticle. Primary conidia uninucleate, obovoidal to subcylindrical, or acerose; bitunicate (the outer wall layer detachable from the conidium except at the basal papilla); forcibly discharged through abdominal hole of the host. Two types of secondary conidia borne on a short conidiophore emerging laterally from the primary spore: ellipsoid type secondary conidia and subglobose secondary conidia; both types forcibly discharged. Resting spores forming a compact mass in host abdomen, bright to dark orange, yellow or peach melba; spores spherical to ovoid, with episporium covered by slightly recurved broad spines.

5. Discussion

Our understanding of the genus *Strongwellsea* is now going through a major transition and maturation. Whenever any genus is originally described with only a single species, it is a great challenge to make the best possible judgments about the limits and most appropriate characterization of the genus as opposed to which characters appear to be most appropriate to distinguish additional species. It is only when

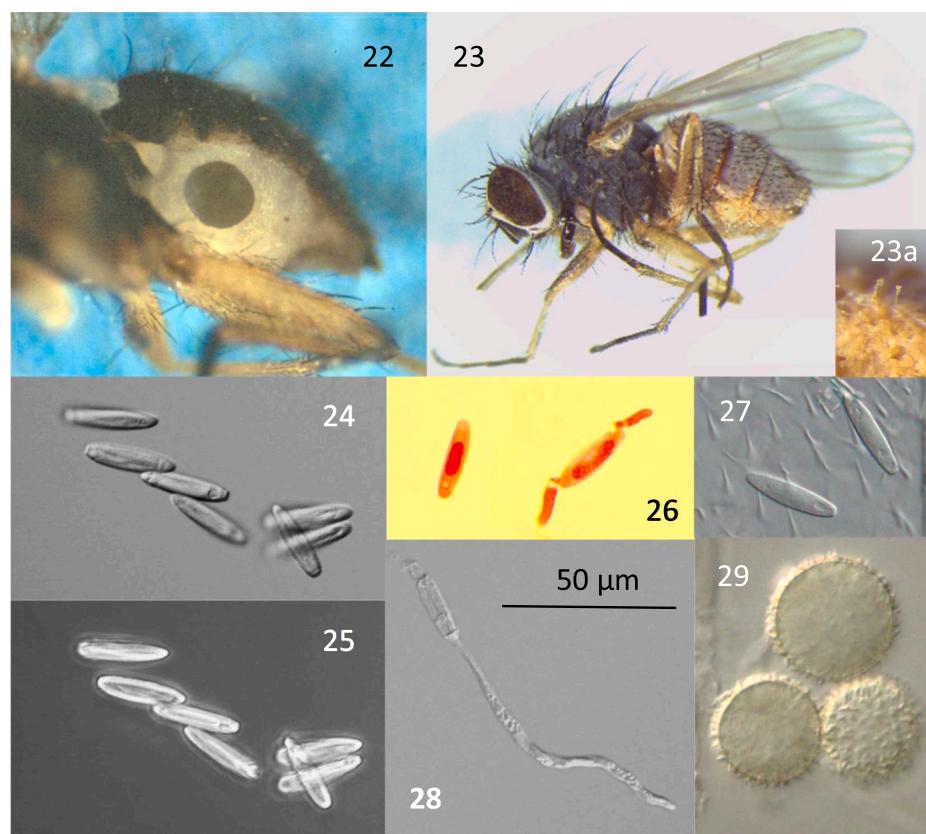


Fig. 22–29. *Strongwellsea acerosa* infected hosts *Coenosia testacea* and spores. (22) Specimen with one abdominal hole, from where primary conidia are discharged. (23) Specimen filled with resting spores. Insert 23a: close up of yellow resting spores. (24) Primary conidia. (25) Primary conidia. (26) Primary conidia, nuclei staining. On the left a recently discharged conidium, on the right is a germinating conidium. (27) Primary conidia on wing. (28) Germinating primary conidium. (29) Resting spores. Light source: Interference Figs. 24, 27, 28 and 29; phase contrast Fig. 25; transmitted light Fig. 26. Scale bar for Figs. 24 - 29 on Fig. 28

additional species are described that the accuracy of these initial choices is either validated or refuted. As the understanding of the biology, distribution, and variations of organisms that can be included in such a genus increases, it is occasionally necessary to reconsider the appropriate circumscriptions of the genus and its species. This emendation of the generic description, addition of new species, and reconsiderations of various aspects of the biology of *Strongwellsea* species (Eilenberg et al., 2020) have, accordingly, been a major goal of these studies.

The first two recognized *Strongwellsea* species show strong host specificities within broad geographical ranges throughout the higher latitudes of the northern temperate zone. Humber (1976) noted that *S. castrans* has a wide geographical distribution affecting species of *Delia*. *Strongwellsea magna* (Humber 1976), on the other hand, was known at that time only from *Fannia canicularis* collected in Berkeley, California, until Eilenberg and Michelsen (1999) found *S. magna* affecting *F. canicularis* as well as three other *Fannia* species in Denmark. It seems likely that *S. magna* has a circumboreal distribution affecting adult *Fannia* species. Until now, the only concerted attempts to collect *Strongwellsea* have been those in Denmark, but the results of those efforts and of all of the other reports in the literature about collections of or attributable to *Strongwellsea* suggest that numerous additional species of this genus still await discovery and description. We recommend that *Strongwellsea* species descriptions should at best address these three main characters: (1) Pathobiology as revealed by host species identity and symptoms on the host such as the size and shape of the abdominal hole, (2) Phenotype as revealed by conidial morphology (size, shape) and if possible resting spore morphology (with special emphasis on the dimensions, color of the spores in mass, details of the episporial spines and, if possible, the number of nuclei per spore), (3) Genotype as revealed by ITS2 and eventually other gene sequences that may be recognized to be especially valuable for the systematics of the entomophthoroid fungi.

Strongwellsea tigrinae is the first species from the genus where the

species description contains information about all spore types: primary conidia, secondary conidia (two types), and resting spores. We recommend all these spore types to be included in species descriptions, and especially we will highlight the importance of the color of the resting spores, which differs between species and add an important character. We are aware that it cannot always be possible to describe the full set of spores. Flies showing symptoms of infections in the conidial stage (hole in abdomen) are easily recognized in the field, while resting spores are tricky to collect; flies must be sampled without showing symptoms, thereafter incubated several days. Further, the percentage of infected flies developing resting spores instead of conidiophores seems low. As is the case of *S. acerosa*, it might happen that secondary conidia are not found, either because the conditions for primary conidia to form secondary conidia are not met, or simply because some species do not form secondary conidia.

Coenosia is a species rich and globally distributed genus of flies belonging to the dipterous family Muscidae. The family Muscidae is very large in itself, containing more than 5,000 species (Kutty et al., 2014). The European fauna alone includes some 70 species from the genus *Coenosia* (Pont, 2005); 31 species are found in Denmark (Michelsen, unpubl. obs.). *Coenosia* belongs to the so-called “hunter flies”, a group of muscids (Coenosiinae) capable of capturing and feeding on other, weaker insects due to modifications of their mouthparts. Most *Coenosia* species are hygrophilous, living in the grass and sedge layer of meadows and fens. The poorly known larvae are also predatory, with most of them living in wet, dark soils (Skidmore, 1985). The present hosts of these newest *Strongwellsea* species, *Coenosia tigrina* and *C. testacea*, are both mesophilous flies typically using the foliage of shrubs in clearings, forest edges and gardens as their “hunting ground”. Especially *C. tigrina*, a relatively large (approx. 6 mm) and robust species, is a voracious hunter of other smaller Diptera. *Coenosia testacea* is a much smaller and more delicate muscid fly allowing just a small abdominal hole to develop and therefore seems to be an unlikely host of *Strongwellsea*. In an ecological context, the species *S. tigrinae* and *S. acerosa* are

placed in the fourth trophic layer, since they parasitize predators. Three different *Strongwellsea* species are now described from *Coenosia* species, and a search for infections in other *Coenosia* species, including *Coenosia attenuata* Stein, which is widely distributed and used in biological control, may very well lead to the discovery of more new *Strongwellsea* species.

Interestingly, *Strongwellsea* shares a set of important characters with the genus *Massospora* (Soper, 1974, Boyce et al., 2019, Macias et al., 2020), even though these genera belong in different subfamilies within the family Entomophthoraceae. Both of these genera show highly specialized, parasitologically sophisticated adjustments to very narrow set of hosts: taxa of adult calypterate (and possible some acalypterate) flies for *Strongwellsea* and taxa of gregarious (but not solitary) cicadas for *Massospora*, which all remain alive during the distribution of their infective conidia. All species in both of these fungal genera are true specialists known from only one or a few other host species from the same genus. The conidia of all species from both genera are dispersed either actively (*Strongwellsea*) or passively (*Massospora*) from a host that is still alive and mobile.

The formation of the characteristic abdominal hole through which the fungus disperses from a living host is a fascinating and unmistakable characteristic of *Strongwellsea* species. These late aspects of the fungal infection, however, tend to obscure how little is truly understood of the development, physiology, pathology, and reproductive details of these remarkable fungi. Studies by Humber (1975, 1976) provided a strong foundation to understand many key aspects of the biology of *S. magna* in *Fannia canicularis*, but similar studies are needed for other species to confirm whether all *Strongwellsea* species show similar or even identical patterns in these all-important aspects of the fungus-host interaction. Many unique aspects of how this fungal genus progresses from its initial infection to the elaboration of the hymenium-lined fungal ball shooting conidia out through the abdominal hole still deserve detailed study. More effort needs to be made to isolate these species *in vitro* to be able to extend studies on the vegetative growth and development of these species and, of course, to support the effort to advance the genomic knowledge of these remarkable fungi. The only assured technique to isolate *Strongwellsea* *in vitro* will be by using vegetative inoculum grown in nutritionally complex liquid media that sustain the naturally protoplastic or hyphal vegetative growth of these fungi (Humber, 1975; Eilenberg et al., 1992).

Acknowledgments

We are grateful to MSc Dorthe Britt Tiwald, who did the sampling of infected *C. testacea* and to director Nils Sættem (Kong Frederik den Syvendes Stiftelse), who allowed insect sampling on the estate areas around Jægerspris. The Carlsberg Foundation granted the Olympus AC70 Provis Microscope.

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