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Piophilidae Distribution in Consideration of Forensic Applications

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Notes on Decomposition

Decomposition as understood by our ancestors have mystical overtones with foggy roots; the reasons behind the beliefs are not readily understood from a twenty-first westerner's perspective. Gases that built up inside the deceased and oozing fluids from the membranes were explained away as flesh eating vampires after a night's feasting (they were bloated and full). Freshly culled meat became source to maggots and flies as it aged, transferring its 'life force' from one being into another (Sachs 2001). A mere century or two ago, Louis Pasteur would be the first westerner to directly link "moulds, mucors and bacteria" to that responsible of decomposition (this was just as the germ theory, that is, organisms smaller than the eye can see exist, was gaining acceptance. Around the same time the acceptance of arthropods playing large roles in decomposition came into scholarly circles but was not accepted among the common person (Sachs 2001). Considering advancements in mathematics and technology are traced back millennia, why did it take us so long to gain further understanding in what was happening with the most basic and routine decompositions? Here, a very large player in flesh decomposition but little understood fly will be investigated to spark interest in those that study the role arthropods play in recycling of organisms, with special interest in recycling of the human body.

Entomology in Forensics

The first translatable written link of entomology application to that of forensics is found in a thirteenth century Chinese detective manual *His Yuan Chi Lu* (The Washing Away of Wrongs), in where the perpetrator of a murder is identified by a cluster of files present on his sickle. The most promising western work on entomological forensics comes in the 1890's by Jean Pierre Megnin, *La faune des cadavres: Application de l'entomologie a la medicine legale* (The fauna of cadavers: Entomological application to legal medicine). This was an attempt by a French scientist to catalog the exact succession of insects and other organisms as a human body decomposed, extending even years after death (Sachs 2001). His work proved to show a very good understanding of succession but failed to address succession in varying habitats and climates. The importance of his ground-laying work cannot be overlooked, for today it is known that although succession of species varies, succession of arthopod families is found to remain constant despite most variables (Horenstein 2010).

The problem Megnin faced was the perceived vulgarity of his work at a time when the dead were not investigated, therefore no scientists, at least in Europe, took his lead. During this time, it was thought so heinous to study human cadavers that there was an entire 'underground' network of grave robbers that would recover the recently dead and 'mad scientists' that would be supplied by such thieves. Not until the latter part of the twentieth century was entomology respected in academia circles enough to openly collaborate on the topic, resulting in an explosion of published work since 1980 and up to the present (Sachs 2001). With the knowledge now gained concerning insects and their role in decomposition, some species still do not hold near enough base of current knowledge as the well-known Calliphorids and Sarcophagids, generally the first to colonize expired fauna. This is where Piophilids will make their grand entrance and add to the knowledge base of forensic entomology; this small fly has emerged from relative obscurity only in the last fifty years but definitive phylogeny of all species is still to be learned.

Skipper Flies

The forensically important fly family of Piophilidae, also known as skipper flies, cheese skippers and bone skippers, are represented by a rather diverse collection of species, comprised of 23 genera and anywhere from 70 to 82 described species and having worldwide distribution (minus Antarctica). These 'skipper' flies have been found feeding on dung, bone, decaying vegetation and fungi (Martin-Vega 2011). By far the best known species of Piophilidae is *Piophilia casei* and it gets its name from 'caseus', Latin for cheese. Caseus also describes the greasy phosphoproteins produced from aging fats. The well-known Piophilids are attracted to proteinaceous substances that exude from the flesh and also flesh by-products such as fats and milk. However, all species listed under Piophilidae are simply categorized on their larval ability to jump or 'skip' when disturbed, presumably in an effort to deter predators. Some larval Piophilids are found favoring the driest of bones like shed antlers rather than the soft tissues of muscle and fatty substances. The differentiation of preferred habitats in Piophilids led some to believe its ancestor resided on the great continent Gondwana (Bonduriansky 1995). Bonduriansky (1995) has demonstrated that two Piophilids categorized in the same genus have vastly different habitats; *Protopiophila latipes* have carrion dwelling larvae while *Protopiophila litigata* have antler dwelling larvae. This large disparity of habitat within related species is just one of many indicators that there is not enough known about this family of 'skipper' flies.

Aiding in this gap of knowledge within the family is also a fundamental taxonomic issue of Piophilids that needs to be addressed. Naming conventions overlapped when first descriptions of Piophilids arose, as it is known as many as four named species to be synonymous with another, for example *Stearibia nigriceps* is also called *Piophila foveolata* by Meigen, 1826 (Kirinoki 2015). Furthermore, recent studies have definitively shown that at least one Piophilid exhibits seasonal polymorphism, with Prochyliza nigrimana exhibiting more melanin in the spring months and less melanin in the summer months of Spain (Martin-Vega 2011).

Piophilids are important even outside of their known detritivore expertise; they are purposefully used in some cheese production in Italy, where they help 'ferment' the protein of milk into cultured cheese.

Because of their usage in cheese production, they have been known to cause enteric myiasis by surviving through the digestive tract and causing lesions in the intestines after eggs and larvae have inadvertently been consumed. Still, relatively little is known about these flies outside of *P. casei*. Most species go unnoticed in nature, where sparse historical data add to their very small size (topping at 5mm) and later arrival in decomposition succession.

The most cited work concerning the family Piophilidae is J. F. McAlpine's article, 'A revised classification of the Piophilidae, including *Neottiophilidae* and *Thyreophoridae* (Diptera: Schizophora)' from *Memoirs of the Entomological Society of Canada*, 1977. McAlpine has at least two other works cataloging Piophilids from South Africa (1978) and Australia (1989), but then two decades pass until another person is seen to dedicate most of their research to Piophilids, and in this case, forensically important Piophilids. Martin-Vega of Universidad de Alcala (Madrid, Spain) has nine out of twelve papers since 2010 focusing mainly on Piophilids. Although there are intermittent individuals adding to the knowledge concerning this family such as Rochefort of McGill University and Prado e Castro of the University of Lisbon, the two most cited individuals are McAlpine and Martin-Vega.

Confusing the already obscure knowledge base of Piophilids are claims like Michelsen's 1983 claim (and subsequent peer reviewed publication) to having rediscovered a once thought extinct Piophilid species, described once 'from memory' by Robineau-Desvoidy in the 1820's. With this 1983 claim comes one more species added to the already cataloged species with no agreeable verification or key produced, outlining the species of *Thyreophora*, identified by Michelsen (1983). Furthermore, there is to be a discrepancy in naming conventions between what most North America taxonomists follow and that followed in Europe, which results in the name most familiar at the time being used in the literature. It is dangerous to put a quantity on the known species of Piophilidae firstly due to the disunity of a naming convention but also due to the lack of confirmation on the presence of species in any particular habitat. For example, *Piophilia casei* is cited so much as the skipper of presence and is so ubiquitous while its cousins like *Piophilia metastigmata* and *Prochyliza spp.* are not equally represented in the literature, even for regions they are known to be endemic. Either recent literature is being used to determine the local Piophilid (which could reasonably result in magnification of reported sightings), existent keys rely on coloration where inter-species variation is known to occur, or *P. casei* has found a way to adapt to a variety of niches that its cousins cannot replicate. Thus the recent work of Rochefort proves ever more pertinent, as she states "a comprehensive phylogenetic analysis and revised classification of the Piophilidae is required" (Rochefort 2015). With the continued work of Rochefort and Martin-Vega, it is reasonable to predict that such revision is in the very near future.

A considerable fraction of the species (about 30%) has been found to hold forensic value. Piophilids frequent decomposing remains starting in the latter part of bloat stage, peaking in the advanced decay stage and tracing the skeletonized stage of decomposition, considering the four to five stages of decomposition now in common observation: fresh, bloat, decay (active / advanced) and remains. Multiple studies have even witnessed Piophilidae species showing up to cadavers as early as the fresh stage, but then disappearing only to show up later. Piophilids are likely out competed by early successors at this stage, and even if eggs are laid, they hold little chance of developing before falling prey to opportunistic scavengers such as ants (Martin-Vega 2011).

Skipper Temperature Preference

Piophilids are surprisingly cold hardy, entering diapause as warm as 17°C and surviving until at least 11°C (Marchenko 2001). Temperatures 22°C and warmer seem to have an adverse effect on *Stearibia nigriceps* (with unknown humidity) (Matuszewski 2014) and adverse effects were also noted to begin between 25°C and 28°C for *Piophilia casei* at 5% relative humidity (Russo 2006). In fact, colder climates may be the niche exploited by Piophilids to keep from being out competed by the bigger, faster moving flies of more temperate regions. Martin-Vega points out there might be more than temperature preference to consider, noting that *Liopiophila varipes* are confined to high elevations in central Spain (Martin-Vega 2011).

Documented Skipper Distribution

Upon a literature survey, a total of 20 Piophilidae species were encountered and distribution of each species was noted. It is important to understand the amount of synonyms used worldwide when referring to some Piophilids. *Piophila foveolata* is referred to in east Europe and Russia while *Stearibia nigriceps* is referred to in the west, while both names are considered to describe the same species (Martin-Vega 2011). Maurizio points out another group of synonyms used; in 1830, Robineau-Desvoidy designated a piophilid as *Thyreophora anthropophaga* but by 1903, it had been moved to the genus Centrophlebomyia by Hendel in an effort to properly display phylogeny (Maurizio 2013). Subsequently, the genus *Parapiophila* is determined a synonym of *Arctopiophila* and *Allopiophila* per McAlpine and Hendel (Martin-Vega 2011) while *Liopiophila* is thought of as a synonym for *Prochyliza* according to Prado e Castro (2010). Another hindrance to literature review are cultural barriers; not only are languages a hindrance to exhaustive searches but also the adoption of western cataloging and reporting in shared peer-reviewed journals.

Table 1. Piophilidae distribution by species

Genus Species	Location	Grid Coordinates	Substrate	Reference
Piophila casei	Melbourne, Australia	37.8°S 144.9°E	puparia adhered to socks	Archer 2005
	Central Argentina	37.2°S 67.4°W	human remains	Martin-Vega 2011
	Cordoba, Argentina	31.4°S 64.2°W	pig carrion	Horenstein 2010
	Campinas, Brazil	22.9°S 34.6°W	pig carrion	Carvalho 2000
	Recife, Brazil	8.2°S 34.6°W		Vasconcelos 2015
	Goiana, Brazil	7.4°S 35.0°W		Vasconcelos 2015
	Saskatchewan, Canada			Sharanowski 2008
	Costa Rica			Carvalho 2000
	Germany			Baumjohann 2013
	Oyama City, Japan	35.3°N 139.0°E	human remains	Kirinoki 2015
	Malaysia	3.1°N 101.7°E	human remains	Martin-Vega 2011
	Lisbon, Portugal	38.7°N 9.1°W		Prado e Castro 2010
	Spain			Martin-Vega 2011
	South Africa			Braack 1986
	Thailand			Sukontason 2001
	Colorado, United States			de Jong 1999
	Hawaii, United States			Early 1986
	Louisiana, United States			Watson 2008
	Tennessee, United States		canine carrion	Reed 1958
Piophila metastigmata	South Africa	30.0°S 25.0°E		Martin-Vega 2011
	Central Spain	40.0°N 4.0°W	pig carrion	Martin-Vega 2011
	Portugal	38.7°N 9.2°W	human remains	Martin-Vega 2011
	Lisbon, Portugal	38.7°N 9.1°W		Prado e Castro 2012
	South Africa			Braack 1986
Stearibia nigriceps	British Columbia, Canada			Anderson 1995
Synonym:	Manitoba, Canada			Gill 2005
Piophila foveolata	New Brunswick, Canada			Michaud 2010
	Colombia			Grisales 2010
	Troy, Czechoslovakia	50.7°N 14.2°E	pig carrion	Hrdrnova 2013
	Central France	46.0°N 2.0°E		Martin-Vega 2011
	Germany			Fiedler 2008
	Greenland			Rochefort 2015
	India			Sathe 2013
	Venice, Italy	45.4°N 12.3°E	human remains	Turchetto 2001
	Kanaus, Litnuania	54.0°N 23.0°E	variable size mammals	Marchenko 2001
	Biedrusko, Poland	52.0°N 17.0°W	pig carrion	Matuszewski 2014
	Coimbra, Portugal	40.2°N 8.4°W		Prado e Castro 2010
	Leningrad, Russia	59.0°N 30.0°E	variable size mammals	Marchenko 2001
	Spain			Martin-Vega 2011
	United States			Rochefort 2015
	Louisiana, United States			Watson 2003
	Tennessee, United States		canine carrion	Reed 1958
	Virginia, United States	37.0°N 79.9°W		Tabor 2004
Centrophlebomyia anthropophaga Synonym: *Thyreophora anthropophaga*	Paris, France	48.5°N 2.2°E		Maurizio 2013
	Kashmir, India	33.5°N 76.2°E		Michelsen 1983
Centrophlebomyia furcata	Finca el Castanar, Spain	37.9°N 3.3°W	Variable size mammals	Martin-Vega 2011
	Israel	31.0°N 35.0°E		Maurizio 2013
Centrophlebomyia grunini	Amur region, Russia	54.5°N 127.8°E		Maurizio 2013

Centrophlebomyia orientalis	Kashmir, India	33.5°N 76.2°E		Maurizio 2013
Liopiophila varipes	Canada			Rochefort 2015
	New Brunswick, Canada			Michaud 2010
	Troy, Czechoslovakia	50.7°N 14.2°E	pig carrion	Hrdrnova 2013
	Germany			Fiedler 2008
	Greenland			Rochefort 2015
	Oyama City, Japan	35.3 °N 139.0°E	human remains	Kirinoki 2015
	Coimbra, Portugal	40.2°N 8.4°W		Martin-Vega 2011
	Central Spain			Prado e Castro 2010
	United States	40.0°N 4.0°W		Rochefort 2015
Mycetaulus bipunctatus	Canada			Rochefort 2015
	Greenland			Rochefort 2015
	United States			Rochefort 2015
	Louisiana, United States			Watson 2008
	Mexico			Rochefort 2015
Mycetaulus subdolus	Canada			Rochefort 2015
	Vermont, United States	44.2°N 72.6°W		Rochefort 2015
Parapiophila atrifrons	Canada			Rochefort 2015
	Alaska, United States			Rochefort 2015
	Western United States			Rochefort 2015
Parapiophila flavipes	Canada			Rochefort 2015
	Alaska, United States			Rochefort 2015
Parapiophila vulgaris	Canada			Rochefort 2015
	Troy, Czechoslovakia	50.7°N 14.2°E	pig carrion	Hrdrnova 2013
	Central United States			Rochefort 2015
	Germany			Fiedler 2008
	Greenland			Rochefort 2015
	Poland			Matuszewski 2008
Prochyliza azteca	Costa Rica			Jirón 1981
Prochyliza brevicornis	Canada			Rochefort 2015
	United States			Rochefort 2015
	Durango, Mexico	24.9°N 104.9°W		Rochefort 2015
Prochyliza nigrimana	Canada			Rochefort 2015
	Greenland			Rochefort 2015
	Lisbon, Portugal	40.2°N 8.4°W		Prado e Castro 2010
	Central Spain	40.0°N 4.0°W	variable carrion	Martin-Vega 2011
	Tennessee, United States		canine carrion	Reed 1958
Prochyliza xanthostoma	Canada			Rochefort 2015
	Illinois, United States		small mammals	Johnson 1975
	Louisiana, United States			Watson 2003
	Tennessee, United States		canine carrion	Reed 1958
	Virginia, United States	37.0°N 79.9°W		Tabor 2004
Protopiophila contecta	Tochigi City, Japan	36.2°N 139.4°E	human remains	Kirinoki 2015
Protopiophila latipes	New Brunswick, Canada			Michaud 2010
	Ontario, Canada	45.8°N 78.7°W	rodents	Bonduriansky 1995

Protpiophila latipes (cont.)	Troy, Czechoslovakia	50.7°N 14.2°E	pig carrion	Hrdrnova 2013
	Germany			Fiedler 2008
	Coimbra, Portugal	40.2°N 8.4°W		Prado e Castro 2010
	Northern United States			Rochefort 2015
	Illinois, United States		small mammals	Johnson 1975
	Tennessee, United States		canine carrion	Reed 1958
Protopiophila litigata	Ontario, Canada	45.8°N 78.7°W	antlers	Bonduriansky 1995
Thyreophora cynophila	Galve de Sorbe, Spain	41.2°N 3.2°W	variable size mammals	Martin-Vega 2011
	Central France	46.0°N 2.0°E	large mammal carcasses	Martin-Vega 2011

Concluding Remarks

Proper identification of Piophilid species worldwide is an important component to accurate identification of arthropod succession and especially has relevance in medico-legal investigations. With the multiple synonyms employed in naming Piophlilidae members and unrecognized cases of polymorphism, skipper flies are being misidentified more times than necessary. Further research into this forensically important family of flies is needed and an extensive investigation (and revision where necessary) into the conventional naming of each genus, clarifying and removing synonymy where relevant.

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