

UPDATED RECORDS OF PHILIPPINE ENTOMOPHTHORALES¹

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¹ Based on the lecture delivered by the author in 2001 as recipient of the Philippine Agriculture and Resources Research Foundation, Inc. (PARRFI) Professorial Chair at the Visayas State University. Information on this group of fungi after 2001 were added to give an updated review on this topic.

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ABSTRACT

Many species of fungi under the order Entomophthorales are important pathogens of arthropods often resulting in high mortalities and natural control of field populations of insects and mites. Recognizing the presence of these fungi may aid in developing better management strategy to control these pests. This paper is a review of the various studies on Philippine Entomophthorales.

Early and recent identification of entomopathogenic fungi was done in collaboration with foreign scientists. A total of 19 species under 9 genera are now included in the record of Philippine Entomophthorales. Of these species, the most common are *Neozygites fresenii* (Nowakowski) Remaudiere & Keller infecting aphids and *Zoophthora radicans* (Brefeld) Batko infecting several insect species. Six species under Genera *Batkoa*, *Entomophthora*, *Furia*, and *Neozygites* are cited as recently described. *Batkoa* (*Entomophaga*) *bukidnonensis* (Villacarlos & Wilding) Villacarlos is a new combination.

The potential of *Batkoa amrascae* and *N. fresenii* as biological control agents was discussed in relation to their biological characteristics. Epizootiology of these species was also described together with the *N. heteropsyllae*, *Z. radicans*, *Pandora blunkii* (Lakon & Zimm.) Humber. Weather conditions and host density affect the occurrence of epizootics in the insect population. Entomophthorales are highly density-dependent; hence, they remain unnoticed when the insect population is low.

Two isolates of *N. fresenii* differ in their virulence to certain aphid species and in the length of their capilliconidiophore. The possibility of using the latter character to identify these isolates, together with other future research based on information presented, is discussed.

Key words: Entomophthorales, entomopathogenic fungi, biological control

INTRODUCTION

The order Entomophthorales is one of the major groups of entomopathogenic fungi commonly encountered in the field causing mortalities of insects and mites. Most species in this order discharge their conidia with force that aids dispersal of the fungus and facilitates the contamination and eventual infection of nearby healthy hosts. Under natural conditions, they can cause epizootics (epidemic) resulting in drastic reductions in host populations (Wilding & Perry 1980, McGuire et al. 1987, Feng et al. 1991, Hollingsworth et al. 1995). They are mostly host-specific, a feature commonly considered an advantage for potential biological control agents (Wilding 1981, Glare & Milner 1991). Further, they multiply rapidly and are able to survive unfavorable conditions. Although there are limitations or difficulties in their culture *in vitro* to produce large quantities for field application (Wilding & Latteur 1987), some species have been successfully used for pest control (Milner et al. 1982, Ekesi et al. 2005 and Steinkraus et al. 2002).

Keller (2007a) recently recorded four families, three subfamilies, 16 genera and 232 species, including the nine new species described by Keller (2007b), of arthropod-pathogenic Entomophthorales worldwide. Only a few of these have been reported in the Philippines. Gabriel (1968 & 1970) documented the early records on entomopathogens in the Philippines where he included at least 12 species of Entomophthorales. Many of the species were identified and notes taken by foreign mycologists such as: Patouillard, Sydow, Rehm, Saccardo, Bresadola, Hennings and Petch (Gabriel 1968). Among the species recorded were: *Entomophaga grylli*, *Neozygites fresenii*, *Conidiobolus coronatus*, *Entomophthora muscae*, *Zoophthora radicans* (*Entomophthora spaerosperma*) and other unidentified species placed under the genus *Entomophthora*. *Z. radicans* was also noted by Velasco in 1983.

In 1986, there occurred an infestation of the jumping louse, *Heteropsylla cubana* Crawford, causing severe defoliation of ipil-ipil (*Leucaena leucocephala* [Lam.] de Wit). Many psyllids infected with Entomophthorales were found. Four species were described in collaboration with Dr. Neil Wilding of the Rothamsted Research at U.K.: *Entomophthora philippinensis*, *Furia* (*Erynia*) *triangularis*, *Batkoa* (*Entomophaga*) *bukidnonensis* (a new combination) and *Neozygites heteropsyllae* (Villacarlos & Wilding 1994). In the same year, Rombach et al. (1994) of the International Rice Research Institute (IRRI) published information on the pathogens of rice insects which included three species infecting leafhoppers and planthoppers: *Pandora delphacis*, *Zoophthora radicans* and *Conidiobolus coronatus*. Two other species were described in collaboration with Dr. Siegfried Keller of Switzerland: *Batkoa amrascae* infecting the cotton leafhopper *Amrasca biguttula* (Ishida) (Villacarlos & Keller, 1997) and *Entomophthora leyteensis* infecting the whitefly *Tetraleurodes acaciae* Quaintance (Villacarlos et al. 2003). Villacarlos & Mejia (2004) recorded 9 genera of Entomophthorales representing 34% of the

entomopathogenic fungi they collected in 1998-2001. Among these, only *N. fresenii*, *B. amrascae*, *Pandora blunkii* and *Zoophtora radicans* have been superficially studied in the laboratory and in the field (Mejia et al. 2000, Subere 2003, Villacarlos and Keller 1997, and Reithmacher et al. 1992). Before any of these can be utilized to control arthropod pests it is essential to have enough knowledge on the biological characteristics of the different species found in the Philippines. Information on entomopathogen-insect and plant host interaction, including their ecological attributes or requirements, is also important (Eilenberg and Pell 2007, Shah et al. 2004, Eilenberg & Michelsen 1999 and Hajek & Leger 1994).

This paper is mainly based on the PARRFI Professorial Chair lecture by the author in 2001 at the Visayas State University (VSU, formerly Visayas State College of Agriculture, ViSCA). In view of the recent developments in this field, some findings have been incorporated in this paper to provide a more complete picture of the entomophthoralean species in the Philippines. Some specific examples and data from published and unpublished works, together with interesting observations of the author, are also included. The general objective of this paper is to give information on the diversity and importance of this group of fungi. Specifically, this paper aims to give an overview on the results of the various researches conducted on Philippine Entomophthorales and discuss the research prospects in the country.

CHARACTERISTICS OF ENTOMOPHTHORALEAN

GENERA/SPECIES REPRESENTED IN THE PHILIPPINES

The taxonomy of arthropod-pathogenic Entomophthorales had been subjected to many changes in the past (Ben Ze'ev & Kenneth 1982, Humber 1982 and 1989, Balazy 1993). Recently, Keller (2007) based on Keller and Petrini (2005), summarized the systematics, taxonomy and identification of these fungi. Included are families: Ancylistaceae, Entomophthoraceae, Meristacraceae and Neozygitaceae. The family Entomophthoraceae is further divided into three subfamilies with their corresponding genera: Entomophthoroideae include: *Batkoa*, *Entomophaga*, *Entomophthora* and *Eryniopsis*; Erynioideae include: *Erynia*, *Furia*, *Orthomyces*, *Pandora*, *Strongwellsea* and *Zoophtora*; and Massosporoidea include *Massospora* and *Tarichium*. Except for Meristacraceae, representatives of the other three families have been recorded in the Philippines (Villacarlos & Mejia 2004).

The succeeding sections describe the characteristics of the different genera and species given in Table 1. The descriptions of the genera followed that of Humber (1997) and Keller (2007), if not stated otherwise.

Table 1. List of insect-pathogenic Entomophthorales in the Philippines.

Family (Subfamily)/Species	Insect Host/ Plant where collected	Place/Date (reference)*
Entomophthoraceae Winter (Entomophthoroideae)		
1. <i>Batkoa amrascae</i> S. Keller & Villacarlos	<i>Amrasca biguttula</i> Ishida (Hemiptera: Cicadellidae) on: <i>Solanum melongena</i> L.(Solanaceae) & <i>Abelmoschus esculentus</i> (L.) Moench., (Malvaceae) <i>Amrasca ricei</i> Dwarakowska & Pawar (Hemiptera: Cicadellidae) on: <i>Vigna</i> <i>sesquipedalis</i> (L.) Fruw., (Fabaceae)	VSU, Baybay, Leyte 1993 (1) Aborlan, Palawan 15.V.1999 VSU, Baybay, Leyte 5.XI.2000 (2)
2. <i>Batkoa apiculata</i> (Thaxter) Humber	<i>Cicadellid</i> sp. (Hemiptera: Cicadellidae) on: <i>Saccharum</i> <i>spontaneum</i> L. (Poaceae) Unidentified planthopper (Hemiptera: Delphacidae) on: <i>Oryza sativa</i> L. (Poaceae)	VSU, Baybay, Leyte 15.VII.1999 (2) Butuan City, Agusan del Norte 22.IX.1999 (2)
3. <i>Batkoa bukidnonensis</i> (Villacarlos & Wilding) Villacarlos	Ipil-ipil psyllid, <i>Heteropsylla</i> <i>cubana</i> Crawford (Hemiptera: Psyllidae) on: <i>Leucaena</i> <i>leucocephala</i> (Lam.) de Wit (Fabaceae)	Bukidnon 8.III.1988 (3)
4. <i>Entomophaga grylli</i> (Fres.)Batko	<i>Locusta migratoria manilensis</i> Meyen (Orthoptera, Acrididae) <i>Ailopus tamulus</i> Fabricius (Orthoptera: Acrididae) <i>Attractomorpha</i> sp.(Orthoptera: Acrididae) Undetermined grasshopper (Orthoptera: Acrididae) on <i>Parashorea malaanonan</i> (Blco.) Merr., Dipterocarpaceae Undetermined grasshopper (Orthoptera: Acrididae) on <i>Ludwigia octovalvis</i> (Jacq.) Raven, Onagraceae	College, Laguna 1913 (4) College, Laguna 1969 (4a) College, Laguna 1969 (4a) VSU, Baybay, Leyte 4.XII.1998 (2) VSU, Baybay, Leyte 13.II.2001

Table 1 continued...

Family (Subfamily)/Species	Insect Host/ Plant where collected	Place/Date (reference)*
5. <i>Entomophthora leyteensis</i> Villacarlos & S. Keller	Whitefly, <i>Tetraleurodes acaciae</i> Quaintance (Hemiptera, Aleyrodidae) on <i>Gliricidia sepium</i> (Jacq.) Walp., (Fabaceae)	Inopacan, Leyte, 5.VII.1998 (5)
6. <i>Entomophthora muscae</i> (Cohn) Fresenius	Rice whorl maggot, <i>Hydrellia philippina</i> Ferino (Ephydriidae: Diptera)	College, Laguna 1965 (4)
7. <i>Entomophthora philippinensis</i> Villacarlos & Wilding	Ipil-ipil psyllid, <i>Heteropsylla cubana</i> Crawford (Hemiptera: Psyllidae) on: <i>Leucaena leucocephala</i> (Lam.) de Wit (Fabaceae)	Bukidnon 8.III.1988 (3)
(Erynioideae)		
8. <i>Furia (Erynia) triangularis</i> (Villacarlos & Wilding) Li, Fan & Huang	Ipil-ipil psyllid, <i>Heteropsylla cubana</i> Crawford (Hemiptera: Psyllidae) on: <i>Leucaena leucocephala</i> (Lam.) de Wit (Fabaceae)	VSU, Baybay, Leyte 3.II.1989 (3, 6)
9. <i>Orthomyces</i> sp.	Whitefly (Hemiptera, Aleyrodidae) on <i>Tithonia diversifolia</i> (Hemsl.) Gray (Asteraceae)	La Trinidad, Benguet 8.V.2000 (2)
10. <i>Pandora (Erynia) blunkii</i> (Lakon & Zimmermann) Humber	<i>Plutella xylostella</i> L. (Lepidoptera: Plutellidae) on <i>Brassica napus</i> var. <i>chinensis</i> L. (Brassicaceae)	La Trinidad, Benguet 1992 (7)
11. <i>Pandora delphacis</i> (Hori) Humber	<i>Nephotettix cincticeps</i> Uhler (Hemiptera: Cicadellidae) on rice <i>Oryza sativa</i> L. (Poaceae)	ARSEF 458-461, 478-479

Table 1 continued...

Family (Subfamily)/Species	Insect Host/ Plant where collected	Place/Date (reference)*
12. <i>Pandora myrmecophaga</i> (Turian & Wuest in Humber) S. Keller	Ants (Hymenoptera: Formicidae) on: <i>Hopea foxworthyi</i> Elm. (Dipterocarpaceae) and <i>Schizostachyum lumampao</i> (Binco) Merr. (Poaceae) Ant (Hymenoptera: Formicidae) on <i>Abelmoschus esculentus</i> (L.) Moench., (Malvaceae)	VSU, Baybay, Leyte, 7.V.2000 (2) VSU, Baybay, Leyte, 27.XI.1998 (2)
13. <i>Pandora (Erynia) neoaphidis</i> (Rem. & Henn.) Humber	<i>Sitobion ibarae</i> Matsumura (Hemiptera: Aphididae) on <i>Rosa</i> sp.	La Trinidad, Benguet 7.V.1999 (2)
14. <i>Zoophthora radicans</i> (Brefeld) Batko	<i>Plutella xylostella</i> (L.) (Lepidoptera: Plutellidae) on <i>Brassica oleracea</i> var. <i>capitata</i> L (Brassicaceae) <i>Amrasca biguttula</i> (Ishida) (Hemiptera: Cicadellidae) on <i>Solanum melongena</i> L. (Solanaceae) <i>Nilaparvata lugens</i> (Stal.) (Hemiptera: Delphacidae) on <i>Oryza sativa</i> L. (Poaceae) Mirid bug (Hemiptera: Miridae) on <i>Lagenaria siceraria</i> (Mol) Standl. (Cucurbitaceae) Undetermined fly (Diptera) on <i>Lycopersicon lycopersicum</i> (L.) Karst. (Solanaceae) Undetermined fly (Diptera) on <i>Phaseolus vulgaris</i> L. (Fabaceae) Undetermined fly on <i>Pisum sativum</i> L. (Fabaceae) <i>Myzus persicae</i> Sulzer (Hemiptera: Aphididae) on <i>Brassica oleracea</i> var. <i>capitata</i> L (Brassicaceae)	Baguio City 1967 (4) La Trinidad, Benguet, 1992 (7) Bagbag II, Cebu City 13.II.1998 (2) Valencia, Ormoc City, 4.IX.1998 (2) Gaas, Ormoc City 18.VIII.1999 (2) Gaas, Ormoc City, 18.VIII.1999 (2) La Trinidad, Benguet 8.V.2000 (2) La Trinidad, Benguet 2.V.2000 (2a) La Trinidad, Benguet 7.V.2000 (2)

Table 1 continued . . .

Family (Subfamily)/Species	Insect Host/ Plant where collected	Place/Date (reference)*
Neozygita Ben-Ze'ev & Kenneth in Ben-Z'ev, Kenneth & Uziel		
15. <i>Neozygites fresenii</i> (Nowakowski) Remaudiere & S. Keller	<p><i>Aphis sacchari</i> (Zehntner) (Hemiptera: Aphididae) on caged sugar cane, <i>Saccharum officinarum</i> L. (Poaceae)</p> <p><i>Aphis craccivora</i> Koch (Hemiptera: Aphididae) on the following (Fabaceae):</p> <p><i>Vigna sesquipedalis</i> (L.) Fruw.</p> <p><i>V. radiata</i> (L.) Wilc.</p> <p><i>Arachis hypogaea</i> L.</p> <p><i>Gliricidia sepium</i> (Jacq.) Walp.</p> <p><i>Psophocarpus tetragonolobus</i> (L.) D.C.</p> <p><i>Aphis citricola</i> van der Goot (Hemiptera: Aphididae) on: <i>Chromolaena odorata</i> (L.) King & Rob. (Asteraceae)</p> <p><i>Aphis gossypii</i> (Glover) (Hemiptera: Aphididae) on <i>Solanum melongena</i> L. (Solanaceae) and <i>Calocasia esculenta</i> (L.) Schott (Araceae)</p> <p><i>Aphis spiraeicola</i> Patch (Hemiptera: Aphididae) on <i>Mikania cordata</i> (Burm. F.) B.L. Rob. (Asteraceae)</p> <p>Undetermined aphids on <i>Theobroma cacao</i> L. (Sterculiaceae)</p>	<p>College, Laguna 1932 (4)</p> <p>VSU, Baybay, Leyte 31.I.1998 (2)</p> <p>Inopacan, Leyte 1.II.1999 (2) Sto. Turibio, Lipa City 18.V.1999 (2)</p> <p>VSU, Baybay, Leyte 27.II.1998 (2)</p> <p>VSU, Leyte 3.VII.1998 (2)</p> <p>Bago-Oshiro, Davao 31.X. 1998 (2) Inopacan, Leyte 19.III.2000 (2) Catbalogan, Samar 14.IX.2000 (2)</p> <p>Catbalogan, Samar 14.IX.2000 (2)</p> <p>Bago-oshiro, Davao 31.X.1998 (2) Sta. Rita, Samar 14.IX.2000 (2)</p> <p>Bago-oshiro, Davao 31.X.1998 (2)</p> <p>VSU, Baybay, Leyte 5.VII.1999 (2)</p> <p>VSU, Baybay, Leyte 20.IV.1999 (2)</p> <p>VSU, Baybay, Leyte 22.I.1998 (2)</p> <p>Inopacan, Leyte 9.III. 2000 (2)</p>

Table 1 continued...

Family (Subfamily)/Species	Insect Host/ Plant where collected	Place/Date (reference)*
16. <i>Neozygites fumosa</i> (Speare) Remaudiere & S. Keller	<i>Coccidohystrix insolita</i> (Green) (Hemiptera: Pseudococcidae) on Solanaceae: <i>Solanum</i> <i>melongena</i> L. & <i>Sida</i> <i>rhubifolia</i> L.	VSU, Baybay, Leyte 1994 (9)
17. <i>Neozygites heteropsyllae</i> Villacarlos & Wilding	Ipil-ipil psyllid, <i>Heteropsylla</i> <i>cubana</i> Crawford (Hemiptera: Psyllidae) on <i>Leucaena leucocephala</i> (Lam.) de Wit (Fabaceae)	VSU, Baybay, Leyte 29.I. 1990 (3) Guisad, Baguio City 9.V.2000 (2) Gaas, Baybay, Leyte 18.XII.2000 (3) VSU, Baybay, Leyte 12.II.2001 (3)
18. <i>Neozygites</i> nr. <i>microlophi</i> S. Keller	<i>Metatrichosiphum</i> <i>tenuicarpus</i> (Okajima) (Hemiptera: Aphididae) on <i>Castanopsis philippinensis</i> (Blco.) Vidal (Fagaceae)	Baguio City 8.V.1999 (2)
Ancylistaceae Fisher		
19. <i>Conidiobolus coronatus</i> (Constantin) Batko	<i>Nilaparvata lugens</i> (Stal.) (Hemiptera: Delphacidae) on <i>Oryza sativa</i> L., Poaceae <i>Inazuma dorsalis</i> (Motschulsky) (Hemiptera: Cicadellidae) <i>Nephotettix impecticeps</i> (Ishihara) (Hemiptera: Cicadellidae)	IRRI, Los Banos 1994 (8) College, Laguna 1965 (4) College, Laguna 1965 (4)

* Reference: (1) Villacarlos & Keller, 1997 (2) Villacarlos & Mejia, 2004 (2a) Villacarlos & Mejia, 2001 (3) Villacarlos & Wilding, 1994 (4) Gabriel, 1968 (4a) Gabriel, 1970 (5) Villacarlos et al. 2003 (6) Li et al., 1998 (7) Riethmacher, et al 1992 (8) Rombach, et al. 1994 (9) Villacarlos, 2000

FAMILY ENTOMOPHTHORACEAE

Subfamily Entomophthoroideae

Members of the Entomophthoroideae have unbranched conidiophores and conidia are bi- to multinucleate (Keller 2007). Except for *Eryniopsis*, the other three genera are represented as follows:

1. Genus *Batkoa* Humber

The conidiophores of species under *Batkoa* are simple and conidiogenous cells are found on simple conidiophores that have the tendency for neck-like apical narrowing immediately below the conidium. Rhizoids (occasionally present) are 2-3 times thicker than vegetative hyphae or conidiophores, occurring singly with discoid terminal holdfast (Humber 1989).

(a) *B. amrascae* S. Keller & Villacarlos

This was first recorded in 1995 and initially identified as *Entomophaga* sp. infecting *Amrasca bigutulla* but later described as a new species of *Batkoa* (Villacarlos & Keller 1997). The hyphal bodies are irregularly rounded to elongate with 10-11 nuclei while conidiophores are unbranched with 5-12 nuclei and terminally enlarged. The primary conidia measure 20-31 x 17-28 μm , unitunicate, sub-spherical to ovoid, apex rounded, papilla (the basal portion of a conidium by which it is attached to conidiophore and involved in forcible discharge of conidia) rounded or conical, sometimes pointed and with 6-13 nuclei. Primary and secondary conidia are similar in shape, the latter usually smaller. The rhizoids are monohyphal with a diameter of 7-23 μm , emerging ventrally from the thoracic region.

(b) *B. apiculata* (Thaxter) Humber

Cicadellid sp. infected with this fungus is attached on the leaf with about 10-15 monohyphal rhizoids. The primary conidia are sub-spherical (32-37 x 27-32 μm , n=100) with 14-19 nuclei per conidium. The secondary conidia measure 25-32 x 22-29 μm with 8-14 nuclei per conidium (n=50). On the other hand, the infected planthoppers on rice were found attached on the leaf with 10-15 strands of monohyphal rhizoids and had slightly smaller primary conidia (27-37 x 24-31 μm , n=50) with fewer nuclei (10-15, n=50). The hyphae contain 10-20 nuclei.

(c) *Batkoa bukidnonensis* (Villacarlos & Wilding) Villacarlos, com. nov.
Basonym: *Entomophaga bukidnonensis* L. Villacarlos & N. Wilding,
Mycol. Res. 98(2): 159-161. 1994

This is illustrated in Figures 17-25 in Villacarlos & Wilding (1994). The primary conidia are unitunicate, spherical or subspherical with a distinct conical papilla, 33-42 x 27-33 μm and 15-20 nuclei. Rhizoids consist of monohyphal strands. Based on the presence of rhizoids it is not an *Entomophaga* species but rather one under *Batkoa* where it is rightly transferred. With this new combination, the structures of *B. bukidnonensis* should be examined again because of the similarities in its characteristics with that of *B. apiculata*. Since the latter species has been reported to be polyphagous (Balazy 1993), it is possible that *B. bukidnonensis* is a synonym of *B. apiculata*.

2. Genus *Entomophaga* Batko

The characteristics of this genus are similar to those of *Batkoa* except that the conidia are formed by direct expansion of the tip of conidiogenous cell not arising from a narrower neck-like extension of the conidiophore (Humber 1989). Rhizoids and cystidia are never formed.

(a) *E. grylli* (Fres.) Batko

Gabriel (1968) first recorded this species infecting *Locusta migratoria manilensis* Meyen. This was also noted on an unidentified acridid that was attached with its forelegs in the hole on the damaged leaf of *Parashorea malaanon*. The body was covered with glistening, granule-like conidia. The measurements of the obovate conidia, 27-37 x 22-25 μm (n = 50), are within the range of published size of the species. There are 13-17 nuclei per conidium.

3. Genus *Entomophthora* Fresenius

The primary conidia are bell-shaped or campanulate with prominent or indistinct apical point and broad, flat basal papilla containing 2-12 (to ca. 40) nuclei. The rhizoids are monohyphal or absent.

(a) *E. leyteensis* Villacarlos & Keller

This species infects a recently introduced whitefly *Tetraleurodes acacia* on *Gliricidia sepium* (Villacarlos et al. 2003). The primary conidia measure 10-17 x 7-15 μm , with distinct or undeveloped apical points and contain 3-5 nuclei. This fungus can cause 8-31% infection of the whitefly population.

(b) *E. philippinensis* Villacarlos & Wilding

Entomophthora philippinensis-infected *Heteropsylla cubana* Crawford is usually firmly attached to the substrate by its proboscis with the wings bearing some deposited conidia surrounded with a halo of mucilage (Villacarlos & Wilding 1994). The primary conidia measure 15-17 x 13-15 μm and contain 2-6 nuclei.

The two *Entomophthora* species mentioned infect Homoptera. The primary conidia are unitunicate, campanulate and surrounded with a mucilaginous halo upon discharge, tip of the papilla most often broad than pointed. Rhizoids are absent in *E. philippinensis* while those of *E. leyteensis* consist of 2-7 monohyphal, simple strands with no holdfast, emerging from the head or thorax.

(c) *E. muscae* (Cohn) Fresenius

This was one of the species determined by Gabriel (1968). It infects adults of the rice whorl maggot, *Hydrellia philippina*, a pest of young rice plants. The primary conidia measure 27-31 x 20-24 μm with 15-20 nuclei (Keller, 2007).

Subfamily Erynioideae

Keller (2007) placed all species of Entomophthoraceae with branched conidiophores and mononucleate conidia under subfamily Erynioideae. Four of six genera in this taxon are represented in the list.

4. Genus *Furia* (Batko) Humber

The primary conidia are ovoid to cylindrical with basal papilla rounded, bitunicate (outer wall layer may separate in liquid mounts). Rhizoids are not thicker than the conidiophores while the cystidia are as thick as the conidiophores, which differentiate *Furia* from *Erynia* and *Pandora*.

(a) *F. triangularis* (Villacarlos & Wilding) Li, Fan & Huang

The first reported new species of *Furia* in the Philippines was *Erynia triangularis* Villacarlos & Wilding that has been synonymized by Li, Fan & Huang, 1998 to *Furia triangularis*. This is a pathogen of jumping lice, *H. cubana*, and illustrated in Figures 10-16 in Villacarlos & Wilding (1994). The primary conidia are mononucleate, bitunicate, obovoid with distinct conical pointed or triangular papilla demarcated with a more or less distinct collar from the body of the spore. The conidia measure 19-22 x 11-14 μm . The rhizoids are few, monohyphal with a simple holdfast and more or less similar diameter as the conidiophores. This last characteristic of the species justifies the new combination given by Li, et al. (1998).

5. Genus *Orthomyces* Steinkraus, Humber & Oliver

This is a recent genus described by Steinkraus et al. (1998) as follows: conidiophores digitately branched at apices; primary conidia have similar characteristics as the rest of the subfamily Erynioideae; secondary conidia either similar to the primary or globose formed directly on top and aligned with the capillary conidiophore; cystidia and rhizoids when present, slightly thicker than vegetative hyphae.

(a) *Orthomyces* sp.

A few whitefly specimens from the wild sunflower (*Tithonia diversifolia*, and not *Helianthus annuus* as indicated by Villacarlos & Mejia 2004) were found infected with *Orthomyces* sp. A white mycelial mat is concentrated on the abdominal region of the host with slight mycelial growth on the head and thorax. The edge of the mycelial mat has several protruding long hair-like structures which turned out to be cystidia varying in length from 28 to 150 μm with a mean of 81 μm ($n=20$) and a diameter of 5-10 or a mean of 7 μm . The insect is attached to the substrate by many fine rhizoids with diameter of 5 to 10 μm , mean of 8 μm ($n=34$). The mean size of the primary conidia is 11 x 6 μm ($n=74$). The fungus is considered an *Orthomyces* species because of its "acorn-like shape" (Steinkraus et al., 1998) but the size of the conidia in the present specimen is slightly smaller than that of *O. aleyrodis* and no capillary conidiophores were observed in the available specimens (Villacarlos & Mejia 2004). This is the first record of *Orthomyces* in the country.

6. Genus *Pandora* Humber

Humber (1989) indicated the following major characteristics of the genus: primary conidia ovoid to cylindrical, obpyriform to obclavate or fusoid, with no strong tendency to show bilateral symmetry (basal papilla may be displaced laterally from spore axis); uninucleate, bitunicate. Cystidia and rhizoids are 2-3 times the diameter of vegetative cells, and the latter with discoid terminal holdfast.

(a) *P. blunkii* (Lakon & Zimmermann) Humber

Pandora blunkii, together with *Zoophthora radicans*, was reported to cause epizootics in the diamondback moth, *Plutella xylostella*, population in the Philippines (Riethmacher et al. 1992). The primary conidia of *P. blunkii* measure 18-21 x 9-10 μm , slightly bent, elongated papilla distinct.

(b) *P. delphacis* (Hori) Humber

Pandora delphacis was recorded on *Nephotettix cincticeps* and included in the Agriculture Research Service Entomopathogenic Fungi (ARSEF 2001) collection of the USDA. According to Rombach et al. (1994) this species can be isolated and cultured on egg-yolk medium and SDA, suggesting that it may be a suitable candidate for biological control. The primary conidia are ovoid to ellipsoidal, 29-36 x 13-18 μm with rather small, convex papillae (Balazy 1993).

(c) *P. myrmechophaga* (Turian & Wuest in Humber) S. Keller

The species *P. myrmechophaga* has been combined with *Erynia* (Keller 1991) and *Zoophthora* (Balazy 1993) in the past until finally reaching the present combination. *P. myrmechophaga* was first observed in an unidentified ant on *Abelmoschus esculentus* in 1998 and was identified then as *Zoophthora radicans*

by Villacarlos & Mejia (2004). This was also found infecting another unidentified ant in the forest in 2000. Infected insects are attached to the leaf of *Hopea foxworthyi* by numerous fine rhizoids concentrated on the thoracic region and hind legs of the host. The mean dimension of the primary conidia (16-20 x 10-12 μm , L/D = 1.6; n = 50) is within the range of published data on *P. myrmecophaga*. This is the first record of the species in the Philippines.

(d) *P. neoaphidis* (Rem. & Henn.) Humber

An epizootic on *Sitobion ibare* Matsumura caused by *P. neoaphidis* was first recorded by Villacarlos & Mejia (2004) in Benguet. Aphids are attached to the leaf of *Rosa* sp. by 6-7 strands of monohyphal rhizoids. The primary conidia measure 22 x 11 μm (20-25 x 10-13 μm , n = 31) and L/D ratio of 2.

7. Genus *Zoophthora* Batko

Primary conidia are ovoid to cylindrical with prominent papilla while secondary conidia either resemble primaries or elongate capilliconidia passively dispersed from capillary conidiophore. Most of the specimens examined had subcylindrical primary conidia and compound rhizoids together with monohyphal ones. Capilliconidia were often observed and capillary conidiophores arose laterally from the primary conidia.

(a) *Z. radicans* (Brefeld) Batko

Z. radicans is one of the species under Entomophthorales that has been recorded from many different host species of various taxonomic groups (Keller 1991). It has been observed infecting a mirid bug, a species of aphid, leafhopper, planthopper and a fly (Table 1). It was also previously reported by Gabriel (1968), which was recorded then as *Entomophthora spaerosperma* Fres. a synonym of *Z. radicans* (Balazy 1993), infecting the diamondback moth, *P. xylostella*, in Baguio. The primary conidia of *Z. radicans* found on *A. biguttula* measure 15-22 x 5-7 μm with L/D = 3 (n = 50). The adult leafminer (Lepidoptera: Gracillariidae) indicated by Villacarlos & Mejia (2004) as infected with this fungus is actually adult Diptera (*Liriomyza* sp., Agromycidae; Colting et al. 2002) whose larvae are leafminers.

A single specimen of aphid with *Z. aphidis* was included in the paper of Villacarlos & Mejia (2004). Closer examination of the data indicates that it could neither be *Z. aphidis* nor *Z. radicans* because the size of the primary conidia is smaller than that of the former but larger than that of the latter. More specimens should be examined to clarify the situation. Therefore, this is placed under *Zoophthora* sp instead of *aphidis*. None of the insects indicated as infected with *Zoophthora* sp., *Erynia* spp. and *Entomophaga* spp., given in Villacarlos & Mejia (2004), are included in this paper.

FAMILY NEOZYGITACEAE

8. Genus *Neozygites* Witlaczil

Keller (1991) gave the following characteristics of the genus *Neozygites*: spherical or rod-shaped hyphal bodies; unbranched conidiophores; spherical to ovate primary conidia with 3-8 nuclei; presence of capilliconidia with typically bent capillary tubes; spherical or ellipsoidal, binucleate, dark brown to black resting spores; cystidia always absent, rhizoids normally absent.

(a) *N. fresenii* (Nowakowski) Remaudiere & S. Keller

Figure 1 in Mejia et al. (2000) and Villacarlos (2000) and Figure 2 in Villacarlos & Mejia (2004) illustrate the various structures of this fungus. This is a common pathogen of at least four aphid species in the Philippines (Table 1). The ranges of dimensions of the different structures from infected *Aphis craccivora* are as follows: primary conidia (15-20 x 13-16 μm), capilliconidia (18-22 x 10-12 μm), length of capilliconidiophore (18-53 μm), and resting spores (28-35 x 20-23 μm), (n = 50).

Neozygites lecanii on *Aphis sacchari* that was included in the list of Gabriel (1968) is actually *N. fresenii* based on the original article of Petch (1931). This was also noted in Villacarlos (2000).

(b) *N. heteropsyllae* Villacarlos & Wilding

The host of this species is *H. cubana*. The various stages of this fungus are illustrated in Figures 1-9 in Villacarlos & Wilding (1994). This was encountered again in Baguio and Leyte in 2000 (Villacarlos & Mejia 2004). The primary conidia measure 20-33 x 7.5-15 μm , n= 63. According to Keller (1997) there are missing structures in the description of Villacarlos and Wilding (1994) that are needed to determine its position in the grouping of species of *Neozygites*.

(c) *N. fumosa* (Speare) Remaudiere & S. Keller

This species was found infecting *Coccidohystrix insolita* infesting eggplant, *Solanum melongena*, and an unidentified mealybug on *Sida rhombifolia* (Figure 1 G-J, Villacarlos, 2000). The mealybugs turned blackish when infected by this fungus. The primary conidia measure 13-18 x 8-10 μm while the resting spores have a diameter of 15-18 μm .

(d) *N. nr. microlophi* S. Keller

The specimen collected from *Metatrichosiphum tenuicorpus* has the following dimensions: primary conidia (22-27 x 12-20, n=38), capilliconidia (25-37 x 12-15 µm, L/D=2-2.4, n=19), capilliconidiophores (30-62.5 µm, n=50) and resting spores (27-35 x 22-27µm, n=50). These measurements are similar to those given by Keller (1991), however, the number of nuclei in the present specimen is 3-4, mostly 4 (n=14) while Keller reported 4-5 (mostly 5) nuclei per conidium.

FAMILY ANCYLISTACEAE

9. Genus *Conidiobolus* Brefeld

Nuclei in primary conidia of this genus ranged from 50 to 100 that do not stain or only weakly in lactophenol-aceto orcein (Keller 1991).

(a) *Conidiobolus coronatus* (Constantin) Batko

The *Entomophthora coronata* (Constantin) Kevorkian infecting different species of mass-reared rice leafhoppers mentioned by Gabriel (1968) is actually *Conidiobolus coronatus*. Coconut milk can be used to culture this fungus (Gabriel & Padua 1974). One application of this species was reported to have controlled the brown planthopper, *Nilaparvata lugens* (Rombach et al. 1994). *C. coronatus* is also believed to exist largely as a widespread soil saprophyte but known to cause disease not only in insects, but also in mammals, including humans (Keller 1987). Hence, one should be careful in handling this fungus.

BIOLOGY AND CULTURE OF ENTOMOPHTHORALEAN SPECIES

The forcible release of conidia by Entomophthorales is an advantage to the species. Released together with the conidium is part of the cytoplasm from the conidiophore that allows it to adhere tightly onto the substrate (King & Humber 1981). Unlike other entomopathogens such as viruses and bacteria that need to be ingested by the host to cause infection, fungi can directly penetrate the host's integument. Thus, these organisms could be very effective against sucking arthropods. In general, when the conidium happens to land on the integument of a susceptible insect it starts to germinate and form penetration peg. The contents of the conidium enter through the penetration peg and the fungus develops and multiplies in the insect body either in the form of hyphal bodies or protoplasts (structures without cell walls). Once the body contents have been depleted the insect dies. The conidiophores break through the soft region of the insect integument like the intersegmental region and grow on the surface of the cuticle. If conditions are favorable (usually high humidity is necessary) the fungus

forms the primary conidia upon emergence of the conidiophores from the insect body. These in turn can form and eject secondary conidia that are usually the same in shape as the primaries but smaller. In some species such as *Orthomyces*, *Zoophthora* and *Neozygites*, capilliconidia are produced (Steinkraus et al 1998 and Keller 1991). These structures are formed at the apex of capillary conidiophores arising from other conidia and differ in shape from the primary, and are not actively discharged. Some species also form resting spores as their survival structures (Matanmi & Libby 1976, Shimazu 1979, Steinkraus & Kramer 1989, Glare et al. 1989 and Keller 2007)

One of the comprehensive studies on the biology of Entomophthorales was done by Brobyn & Wilding (1977). Representative examples of the life cycle of some species are also given by Keller (2007). In the present paper, two examples are cited, that of *Batkoa amrascae* infecting the okra leafhopper *Amrasca biguttula* and *Neozygites fresenii*, a pathogen of *Aphis craccivora*.

***Batkoa amrascae*.** This is one of the entomophthoralean species that can grow well in culture media. Inoculum grown on Sabouraud dextrose agar (SDA) and in liquid medium (20g D-Glucose, 5g yeast extract, 50 ml fresh milk and 450 ml distilled water) used for *Zoophthora radicans* (Pell et al. 1993) successfully infected healthy leafhoppers at 26-28°C room temperature (Villacarlos & Keller 1997). Fungus grown in SDA and in the liquid media caused 19% and 9% mortalities, respectively, of the 268 treated leafhoppers (unpublished data, Villacarlos 1995). Based on slide mounts of insect teased out at six hours interval after inoculation, it took about 44 hr (1.8 days) for the fungus to penetrate and start developing within the insect body. During this period, only a few mycelia dispersed in the hemocoel were present. Long, irregular-shaped hyphal bodies were normally found in live insects 43-65 hours after inoculation. The insect usually died 3-5 days after treatment. The first structures that came out from the insect cadaver were 7-9 strands of monohyphal rhizoids with disc-shaped holdfasts that anchor the host to the leaf. When teased out, the body of the newly dead insects contained hyphae and developing conidiophores. An infected cadaver placed in a humid chamber showed fungal growth along the intersegmental regions within 4 hours, then began to sporulate within another 3-5 hours. Mycelial growth on the body surface usually appeared creamy, but later changed to a whitish glistening mat when about to sporulate. The primary conidia were forcibly released and under favorable conditions, caused infection of healthy leafhoppers. Subere (2003) found that *B. amrascae* produced 26,372 conidia when the infected leafhopper was placed in the dark and only 5,290 when exposed to light under room temperature. In both cases, the maximum number of conidia was produced at 7-13 hours after the initial sporulation. Furthermore, she observed that leafhoppers inoculated in the laboratory produced significantly fewer conidia than field-collected infected insects. According to Glare & Milner (1991) the number of conidia produced can vary greatly and is dependent on the size of the cadaver and the prevailing humidity. Both these factors were not measured by Subere.

Neozygites frezenii. Infection by *N. frezenii* was first noticed on *A. craccivora* infesting *Gliricidia sepium* (Jacq.) Steud. and stringbean, *Vigna sesquipedalis* Fruw., in the Visayas State University in 1994 (Villacarlos 2000). This fungus has not been successfully grown in vitro, thus, it has to be grown in living aphids (Steinkraus et al. 1993). Inoculation results when healthy aphids were allowed to walk on a field of capilliconidia. Brobyn & Wilding (1977), Steinkraus et al. (1993) and Keller (1997) had earlier described and illustrated the biology and life cycle of this species. In the Philippines, Mejia et al. (2000) observed, on squashed mounts of inoculated aphids, that protoplasts were the first fungal structures found in the body cavity of living insect at 36-40 hours post-inoculation. These developed to hyphal bodies filling up the insect body within 48 hours post inoculation. The insect usually died during the next 6 hours after the body cavity had been filled up with elongated hyphal bodies developing into conidiophores. After another 6 hours conidiophores started emerging from the surface of the aphid cadaver and the fungus sporulated during the next 5-6 hours. The primary conidia formed the capilliconidia after 6 hours. The total developmental period of the fungus from inoculation to sporulation lasted about 3.5 days. *N. frezenii* also produces resting spores or zygospores that are formed by conjugating hyphal bodies (Keller 1997). It is uncertain as to what factors contribute to the formation of the structures mentioned but black, soft cadavers filled up with immature and mature resting spores are usually observed towards the end of an epizootic in the field. Protoplasts and resting spores were not observed in *B. amrascae*.

STRAINS OF *Neozygites frezenii* AND APHID SUSCEPTIBILITY

It has been demonstrated that there is considerable variability in both the susceptibility of different aphids and the virulence of different isolates of the fungus (Pell 2007 and Shah et al. 2003). Such information is important for the correct choice of the most virulent isolate to be used to control specific aphid species. *N. frezenii* is again used as example.

In 1994 a study on two strains of *N. frezenii*, one from the U.S.A. (DS isolate) and the other from the Philippines (LV isolate), was conducted at Rothamsted Research, England. Some interesting unpublished findings (Villacarlos et al. 1996) from this research are given below.

Aphis gossypii (Glover) infesting cotton (*Gossypium hirsutum* L.) was the original host of the DS isolate that was provided by Dr. D. C. Steinkraus of the University of Arkansas, USA. The LV isolate came from infected *A. craccivora* Koch infesting stringbean (*Vigna sesquipedalis* L.) in the Visayas State University. In vivo culture of *N. frezenii* was maintained in the laboratory based on the method developed by Steinkraus et al. (1993). The susceptibility of the different aphid species feeding on their natural plant host was evaluated by exposing the aphids overnight (about 14 hours) to the capilliconidia of these isolates. The bioassays

were conducted at 23°C and mortality assessed daily for 6-7 days. Some morphological characteristics of the two isolates were compared to determine whether one could be distinguished from the other.

The relative pathogenicities of the two isolates to both nymphs and adults of the different aphid hosts, namely, *Aphis fabae* Scopoli, *Lypaphis erysimi* (Kaltenbach), *Sitobion avenae* (Fabricius), *Myzus persicae* (Sulzer), *Rhopalosiphum padi* (Linnaeus) and *Acyrtosiphum pisum* (Harris) were similar, with *A. fabae* being the most susceptible resulting in 100 % nymph mortality and 72% adult mortality due to *N. fresenii* infection. On the other hand, *R. padi* and *Ac. pisum* were the least susceptible to the fungus (8-10% nymph mortality and 0-12% adult mortality). The rest of the aphid species had 21-84% and 7-35% nymph and adult mortalities, respectively, when exposed to the isolates. However, the DS isolate was significantly more virulent to *A. gossypii* reared on different hosts (*Vicia faba* L., *Gossypium hirsutum* L. and *Cucurbita* sp.) than the LV isolate. Mortalities ranged from 82 to 92% for nymphs and 88 to 98% for adults when inoculated with the DS isolate, and only 52 to 70% for nymphs and 39 to 67% for adults when inoculated with LV isolate. This may be due to the fact that the original host of the DS isolate was *A. gossypii*. Apparently, the host plant did not affect the susceptibility of the aphid to infection by *N. fresenii*. On the other hand, Mejia et al. (2000) in the Philippines also found *R. maidis* Fitch to be the least susceptible to *N. fresenii* (LV isolate) with only 28% mortality compared to 53.3 % and 40.6% mortalities of *A. citricola* vd Goot and *Brevicoryne brassica* (L.), respectively.

Dimensions of the different structures of the two isolates of *N. fresenii* are similar in all respects (size of primary conidia, capilliconidia and hyphal bodies) except for the significantly longer capilliconidiophore of the DS isolate (57.7 $\mu\text{m} \pm 15.9$, n=58) compared to the LV isolate (28.3 $\mu\text{m} \pm 10.7$, n=53). The capilliconidiophore length was stable for *N. fresenii* measured from specimens taken from *A. gossypii*, *A. fabae*, *A. craccivora*, *Ac. pisum*, *L. erysimi*, *M. persicae*, *R. padi* and *S. avenae* with a range of 53-97 μm for DS isolate and 28-44 μm for the LV isolate.

EPIZOOTIOLOGY

Like human diseases, the occurrence of insect diseases is affected by certain biotic and abiotic factors, but unlike the former where these factors can easily be measured, this is somehow difficult in the case of the tiny insects. Measurement of such factors is part of the science of epizootiology defined as "the field concerned with the study of diseases of animals on the basis of mass phenomena" (Steinhaus & Martignoni 1970). Knowledge on the factors that enhance the development of a disease caused by a particular entomopathogen like the Entomophthorales is relevant to their successful utilization in pest control. One problem involved in epizootiology is the measurement of prevalence, "the number of hosts (or proportion

of hosts at risk) expressing a disease at a given point in time" (Fuxa and Tanada 1987). Eilenberg & Pell (2007) cited two common methods used to assess prevalence: a) sample living individuals and incubate them individually with provision of fresh food and water which has been used in studies of flies, and b) sample dead and live aphids and examine them under the microscope for structures of the fungus under study. The presence of any of the fungal structures is a positive indicator for infection. The second method was used in the examples given below.

1) Entomophthorales in *Heteropsylla cubana* population

Heteropsylla cubana is an introduced species that was first noted in the country in 1979 and caused defoliation of ipil-ipil (*Leucaena leucocephala*) trees in late 1985 (Villacarlos & Wilding 1994). During the psyllid infestation, Villacarlos & Robin (1989) took monthly samples of infested terminal shoots of ipil-ipil from the Agroforestry Demonstration Farm of the Department of Forestry in the VSU. Healthy and diseased insects (those showing fungal growth) were counted and regular epizootics caused by entomogenous fungi were recorded. Of these, the entomophthoralean species accounted for 51% of the infected insects collected. The trimming of *Leucaena* hedgerows and rainfall had direct effect on psyllid population. New shoots encouraged the rapid development of psyllid population while heavy rains washed off the insect. In general, disease prevalence was high when the number of psyllids was 200 or more per shoot. The entomopathogenic fungi were present even at very low level throughout the year. The presence of these entomopathogenic fungi and other natural enemies must have contributed to the reduction and maintenance at a tolerable level of the psyllid population two years after the initial infestation of *L. leucocephala* in the Philippines.

2) *Batkoa amrascae* in *Amrasca biguttula* population

The cotton leafhopper *A. biguttula* is a common pest of okra, *Abelmoschus esculentus* and eggplant, *Solanum melongena*. During heavy infestation it can cause hopper burn. The presence of *B. amrascae* was first observed causing a sporadic and localized epizootic in the leafhopper population in 1993 in the VSU. Percent prevalence was 0-20% in some areas and 0-70% in others (Subere, 1996). Based on a 9-week (June to July) observation the disease prevalence (live and dead infected insects counted per leaf sample) was 10-42% in June but dropped to 1% in July (unpublished data, Villacarlos 1995). In general, infected leafhoppers were observed when the morning temperature was low and the leafhopper number was on the average above 13 per leaf. There was a negative correlation between temperature and the proportion of infected insects ($r = -0.74$, $n = 9$).

3) *Neozygites fresenii* in *Aphis craccivora* population

An experiment conducted by Mejia et al. (2000) indicated the regular occurrence of *N. fresenii* in *A. craccivora* population infesting mungbean (*Phaseolus aureus* Roxb.), bush bean (*Phaseolus vulgaris* L.) and stringbean (*Vigna sesquipedalis* L.). They recorded the percent infected aphids biweekly for five months (July to November, 1995) in the VSU experimental field. Ten live aphids were collected weekly from each of three infested plants randomly selected per row of a given crop, giving a total of 30 aphid samples per week per crop. Each aphid sample was teased out, mounted in lactophenol cotton blue, and examined under a compound microscope for fungal structures as described by Steinkraus et al (1991). The fungus prevalence was less than 30% at the start of the season but increased three folds during the succeeding weeks of observation. The rainfall amounting to over 100 mm in October washed off the aphids including infected ones. However, 67-93% of the survivors were found to be infected with the fungus. Of the 208 winged aphids collected in yellow pan traps set up during the week of legume emergence in the field, 6.7% were found infected (unpublished data, Villacarlos & Mejia 2001). This implies that a source of *N. fresenii* inoculum could have been migrating alates. Steinkraus et al. (1995) observed that *N. fresenii* prevalence rates were higher in *A. gossypii* alates than in apterae during early stages of epizootics. They indicated that this kind of result was expected if migrating infected alates were responsible for initiating epizootics in aphid infested fields. The weather conditions, including moisture levels and wind conditions, play an important role in sporulation and development of *N. fresenii* epizootics (Steinkraus et al. 1996).

4) *Pandora blunckii* and *Zoophthora radicans* in *Plutella xylostella* population

Natural epizootics of *P. (Erynia) blunckii* and *Z. radicans* occurred in populations of diamondback moth (DBM), *Plutella xylostella* (Riethmacher et al. 1992). The fungi can infect over 95% of the larvae and over 70% of the pupae on cabbage (*Brassica oleracea*). Fungus infections first occurred at 6 weeks in larval population development. Infection levels increased up to 95% at the end of the season 2 weeks after the peak of the larval population. The fungi only occurred at a fairly late stage in plant development, i.e. shortly before harvest and before the DBM populations decreased.

There are many factors that influence the ecology of Entomophthorales as pointed out by Eilenberg and Pell (2007). The four examples above, although limited in scope, further show the importance of weather conditions, insect density and initial source of inoculum in the occurrence of entomophthoralean epizootics in insect populations. Entomophthorales are highly density-dependent, hence they remain unnoticed when insect populations are low.

DISCUSSION AND RESEARCH PROSPECTS

There are many species of entomopathogenic Entomophthorales recorded and studied in various parts of the world that are summarized in Keller (2007). Of the 16 genera and 232 species he mentioned, 9 genera and 19 species are found in the Philippines, representing 50% and 8.2% world wide, respectively. This is actually an underestimation because those identified up to generic level (Villacarlos & Mejia 2004, Gabriel & Padua 1981 and Gabriel 1968 & 1970), except *Orthomyces*, were excluded in the list. The arthropod collection of Villacarlos & Mejia (2004) was concentrated in the Visayas and few areas in Luzon and Mindanao thus, a vast area in the country remains unexplored. There must be many more out in the field left undiscovered. Awareness of the existence of these fungi in arthropod populations, hopefully, can help develop interest among researchers to consider these organisms as one component in their research studies in the Philippines, or in the tropics as a whole, where limited information on this topic is available (Hywel-Jones 2002).

The lack of knowledge and unfamiliarity with entomophthoralean-infected insects tend to mask their contribution in the reduction of pest population levels in the field. In 1999 an outbreak of dipteran leafminers, *Liriomyza* spp., in potato field caused severe damage not only on potatoes but also on other crops in Benguet, Northern Philippines (Colting et al. 2002). The pest outbreak was attributed to many factors, one of which was absence or inadequate population of natural enemies. Entomopathogens were not mentioned as one of the natural enemies of the flies. However, Villacarlos and Mejia (2004) recorded *Zoophthora radicans* and an unidentified *Entomophaga* species infecting the adult flies, (*Liriomyza* sp.) to be common in La Trinidad, Benguet in 2000. As cited by Colting et al. (2002), farmers in the area applied a mixture of insecticides and fungicides at close intervals which might have contributed to the killing of the parasitoids and predators of the pest. It is suspected that, together with these natural enemies, entomopathogens were also killed by the fungicides. Since entomopathogenic fungi in general are important mortality factors in insect population, they may as well be a part of the management strategy. Ecological approaches should be considered in management strategies and, if possible, entomopathogenic fungi should be included (Pell 2007). To do this, researchers should be familiar with the presence of these fungi in the insect population.

Data on the bioassay indicate that *Aphis fabae* and *A. citricola* are the most susceptible while *Acyrtosiphum pisum*, *Rhopalosiphum padi* and *R. maidis* are the most resistant to *Neozygites fresenii*. Aphids are the known host of this fungus which is believed to be common especially among species of the genus *Aphis* (Wilding & Brady 1984). For instance, the DS and LV isolates were taken from *A. gossypii* and *A. craccivora*, respectively. In nature, *N. fresenii* has been recorded infecting many species of *Aphis* and other aphid genera (Table 2). It is

Table 2. Recorded aphid hosts of *Neozygites frezenii*

Aphid species	Country	Reference
<i>Aphis citricola</i> van der Goot	Israel United States	Ben-Ze'ev et al., 1984 Steinkraus et al., 1991
<i>A. craccivora</i> Koch	Philippines Benin	Villacarlos & Mejia, 2004 Keller, 1997
<i>A. fabae</i> Scopoli	Switzerland England Poland	Keller, 1991 Brobyn & Wilding, 1977 Balazy, 1993
<i>A. gossypii</i> Glover	Australia Egypt Israel Philippines South Africa	Milner and Holdom, 1986 Keller, 1997 Ben-Ze'ev et al., 1984 Villacarlos & Mejia, 2004 Hatting et al., 1999
<i>A. rumicis</i> L.	Switzerland	Keller, 1991
<i>A. sacchari</i> (Zehntner)	Philippines	Gabriel, 1968
<i>A. spiraecola</i> Patch	Philippines	Gabriel, 1968 and Villacarlos & Mejia, 2004
<i>A. umbrella</i> (Borner)	Israel	Ben-Ze'ev et al., 1984
<i>Brevicoryne brassicae</i> (L)	Israel, Poland, Switzerland	Ben-Ze'ev et al., 1984; Balazy, 1993; Keller, 1991
<i>Hyalopterus pruni</i> Geoffroy	Israel, Poland, Switzerland	Ben-Ze'ev et al., 1984; Balazy, 1993; Keller, 1991
<i>Microlophium evansi</i> (Theob.)	Israel, Poland, Switzerland	Ben-Ze'ev et al., 1984; Balazy, 1993; Keller, 1991
<i>Myzus persicae</i> Sulzer	Israel, Poland, Switzerland	Ben-Ze'ev et al., 1984; Balazy, 1993; Keller, 1991
<i>Neophyllaphis gingerensis</i> Carver	Australia	Milner & Holdom, 1986
<i>Rhopalosiphum padi</i> (L)	Israel, Poland, Switzerland	Ben-Ze'ev et al., 1984; Balazy, 1993; Keller, 1991

uncertain though as to what degree of susceptibility to *N. fresenii* these aphid species have. Information on the susceptibility and on the natural or ecological host range (Keller 2007) is useful because alternate host species can serve as reservoir for *N. fresenii* that can be source of inoculum in non-crop and crop areas (Ekesi et al. 2005).

The black bean aphid, *A. craccivora*, is one of the major pests of vegetable legumes in the Philippines and has been recorded as vector of 14 plant pathogenic viruses (Calilung, 1967 & 1980). The regular presence of *N. fresenii* in *A. craccivora* population can possibly be enhanced to control this aphid species. There is evidence that infection of aphids by *N. fresenii* is initiated by incoming alates (Steinkraus et al. 1995). Increasing the source of inoculum by introduction of *N. fresenii*-infected aphids at the start of the cropping season may enable the fungus to cause early epizootics and reduce the aphid population prior to pod development of the crop. This method had been tried in England where laboratory cultured aphids inoculated with *Pandora (Erynia) neoaphidis* (Remaud. & Henn.) Humber, *Entomophthora planchioniana* Cornu, *Conidiobolus obscurus* (Hall & Dunn) Remaud. and *N. fresenii* were distributed in aphid-infested field legume early in the season (Wilding 1981). The aphid number was reduced to half and collapse of the population occurred one to two weeks earlier than in untreated areas. It is also possible to introduce infected aphids in areas where *N. fresenii* is absent. This fungus was successfully introduced in cotton fields in San Joaquin Valley, California where the fungus is not naturally present (Steinkraus 2002). *N. fresenii*-infected *A. gossypii* "cadavers" (Steinkraus et al. 1993) were placed underside of an aphid-infested cotton leaf on each replicate plant in the released fields. The release of *N. fresenii*-infected aphid cadavers resulted in high infection levels which reached 14% considered imminent for epizootics (Steinkraus 2002). The fungus successfully spread and persisted in the aphid population after its release.

Similarly, *Entomophaga maimaga* Humber, Shimazu & Soper which was initially introduced from Japan to the USA to control *Lymantria dispar* L (Hajek et al. 1990), was successfully introduced in other areas where the fungus is absent. Resting spores of *E. maimaga* produced in one area were collected and transferred to areas where these were needed (Hajek & Wheeler 1994). In their case, it is easy to find the resting spores because these are the overwintering structures of the fungus. Thus, in spring they are sure to find resting spores in the soil. Like *E. maimaga*, *N. fresenii* also forms resting spores, however, it would be essential to find out how to facilitate the formation of these propagules. It is still unknown what exactly triggers the formation of resting spores of *N. fresenii*, although these were mostly observed during the late stages of an epizootic. The production of resting spores by *Zoophthora radicans* was found to correlate with inoculum density (Glare et al. 1988). During an epizootic many infected individuals are normally found in an *A. craccivora* colony, thus inoculum density would be very high in such situation and may explain the formation of resting spores in

the rest of the contaminated aphid colony at the end of the epizootic phase. Simulated situation in the laboratory where aphids are exposed to high inoculum density may be done to produce resting spores for field application.

The morphological difference in *N. fresenii* infecting *A. gossypii* (DS isolate), a strain having long capilliconidiophore, and *A. craccivora* (LV isolate) strain having short capilliconidiophore, could be examined closely. It is possible to divide *N. fresenii* into two strains, *A. gossypii* strain (DS isolate) and *A. craccivora* strain (LV isolate). The length of capilliconidiophore in Steinkraus et. al. (1991) collection of infected *A. gossypii* on cotton was 25-60 μm . Milner and Holdom (1986) also noticed longer capilliconidiophores (77 μm) of *N. fresenii* from the aphid *Neophyllaphis gingerensis* feeding on the shrub *Podocarpus lawrencei* J. D. Hooker and from *Aphis gossypii* (66 μm). These examples may be considered under the *A. gossypii* strain. It is also possible that the *A. craccivora* strain is more common in tropical countries like the Philippines and the *A. gossypii* strain in temperate countries.

The capilliconidiophores of *N. fresenii* on *A. craccivora* in S. Keller collection from Benin had a mean length of 21.3 μm (Keller 1997) while those on *A. fabae* collected in Switzerland had 73 to 220 μm (Keller 1991). Length measurement of capilliconidiophores of *N. fresenii* in specimens from various sources (hosts and locations) may elucidate further the possibility of using this character in determining the existence of different strains. The use of molecular method in identifying strains of this species is possible if the organism can be grown successfully *in vitro*.

Enhancement of insect pathogen prevalence to suppress arthropod pest population requires some effective method for assessing its presence in the population. Measurement of pathogen prevalence in aphids is generally more difficult than in bigger insects. Steinkraus et al. (1991, 1995) developed a method to determine the prevalence of *N. fresenii* in a given aphid population. In their method, all aphids (live and dead) on one-fourth of the leaf area on each leaf were removed, dissected and mounted in lactophenol on a microscope slide. If hyphal bodies were present in live aphids, or if conidiophores and conidia of the pathogen were found on dead aphids, they were considered infected. Mejia et al., 2000, slightly modified this method by considering only the live aphids showing the fungal structures. Based on the biology of the fungus they assumed that those containing the fungus structures will die a few days after collection. This method does not include the "recently dead" aphids; only "infected-still alive" (Eilenberg & Pell, 2007). The major disadvantage of this is underestimation because of individuals missed at an early stage of infection and the possible presence of species other than *N. fresenii*. These factors aside, the main point to establish if this kind of sampling method will be used is the number of live aphids needed to get a more or less unbiased measure of the incidence or prevalence.

In Hyphomycetes, it is common to grow some species like *Metarhizium anisopliae* and *Beauveria bassiana* on grain substrate to produce conidia for field application (Ferron 1981 and Rombach et al. 1986). Such substrates are not commonly used among Entomophthorales. Recently, however, *Pandora delphacis* has been successfully grown on broomcorn millets in China (Mingguang & Yong 2003). It is an important entomopathogen of rice leafhopper and brown planthopper *Nilaparvata lugens* and a good candidate for the control of these rice pests (Rombach et al., 1994). With the availability of a mass production scheme, it may be possible now to use *P. delphacis* for field application to control the said pests. On the other hand, *Batkoa amrascae* that grows very well on SDA and liquid medium used for *Z. radicans* culture (Pell et al. 1993) probably can also grow on cereal media. Available local materials could be tried for possible mass production of both *P. delphacis* and *B. amrascae*. If successful, the fungi then can be used easily for field application like other Hyphomycetes.

There are aspects in the entomopathogen-insect host and host plant interaction that can also be examined. For instance, it has been shown that the pathogen affect the host insects involving their feeding, reproductive or even social behavior (Roy et al. 2006). Further, insect host plants and predators have been reported to affect pathogen transmission and spread of some species of Entomophthorales (Ekesi et al. 2005 and Pell 2007). These are interesting areas to look at not only for entomophthoralean species but for other entomopathogenic fungi as well.

This paper presented some of the important biological, morphological and ecological features of entomopathogenic Entomophthorales found in the Philippines. Hopefully, these pieces of information will encourage conduct of more studies on this interesting group of fungi. The study on Entomophthorales is an open field that entomologists, mycologists and other scientists could explore.

For those who will be interested to work on Entomophthorales or entomopathogenic fungi in general, herbaria of some infected cadavers mentioned in this paper and in Villacarlos & Mejia (2004) are deposited in the Entomology Section of the Philippine National Museum. Some slide mounts of the fungi (if still in good condition) had been sent some years ago to Dr. Richard Humber of the United States.

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