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Short communication

Evolution of *Dicer* and *Argonaute* orthologs in microsporidian parasites

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ABSTRACT

Microsporidia are a group of intracellular parasites which infect animal hosts. The infection can broadly influence the hosts' metabolism, growth as well as immune responses. Recently, a functional RNAi pathway was suggested from the microsporidia parasite *Nosema ceranae*, whereby the gene *Dicer* showed strong impact on spore proliferation. Based on sequenced microsporidian species, the RNAi gene orthologs have only been annotated for a few species. In order to study the selection of RNAi gene *Dicer* and *Argonaute* orthologs from microsporidian genomes, a phylogenetic analysis was performed based on single copy orthologs of 21 microsporidian parasite species. Of the 21 studied parasite species, 11 parasite species maintained *Dicer* and *Argonaute* orthologs, which were further used to build the gene trees. The gene *Dicer* and *Argonaute* orthologs were either both maintained or both lost. The topology structures between the phylogenetic *Dicer*, *Argonaute* and species trees were consistent. The results suggest that the gene *Dicer* and *Argonaute* were selected as a unit, which were selectively maintained/lost during the lineage divergence. The study provides general insights on the selection of RNAi pathway in microsporidian parasites and the evolution of parasitism.

1. Introduction

Microsporidia are an early diverging clade of fungi which comprise a large group of spore-forming unicellular parasites (Capella-Gutiérrez et al., 2012). The genomes of microsporidian parasites are generally compact due to large history as intracellular parasite, ranging between 2Mbp – 15Mbp (Ndikumana et al., 2017). However, an exceptionally large genome of 51 Mbp has also been observed (Desjardins et al., 2015). Classic mitochondria are absent, however tiny mitochondrially derived organelles called mitosomes are maintained (Burri et al., 2006). Microsporidia infections have been reported to inhibit host apoptosis (del Aguila et al., 2006; Martín-Hernández et al., 2017a), as well as suppressing host innate immune response and regulating host metabolism (Antunez et al., 2009; Cuomo et al., 2012). The infection even leads to the death of the host (Hodgkin and Partridge, 2008). Recently, a functional RNAi pathway was suggested from deep sequencing studies of the microsporidian parasite *Nosema ceranae* (Huang et al., 2016b; Huang and Evans, 2016). The RNAi mechanism was also found in another plant infecting fungal parasite *Botrytis cinerea*, where the parasitic microRNAs were used to suppress the host immune responses (Weiberg et al., 2013).

The variant gene regulation mechanism may have substantial impacts on the strategy adopted by the parasites to establish the infection. From the genome point of view many microsporidian parasite species lost their RNAi gene orthologs. The mechanism to drive the selection of RNAi orthologs remains unclear. *Dicer* and *Argonaute* are key genes in RNA induced silencing complex, which involved in small regulatory RNA maturation and degradation of mRNA (Carthew and Sontheimer, 2009). In this study, the orthologs of two key RNAi genes *Dicer* and *Argonaute* were screened through 21 microsporidian parasite species and phylogenetic analyses were performed to provide insights of RNAi genes selection in microsporidian parasites.

2. Material and methods

The protein sequences of 21 microsporidian parasites were retrieved from NCBI and MicrosporidiaDB (Table 1) (https://www.ncbi.nlm.nih.gov/nuccore/AOMW00000000.2/; https://www.ncbi.nlm.nih.gov/nuccore/AEYK00000000.1/) (Campbell et al., 2013; Chen et al., 2013; Corradi et al., 2010; 2007; Cuomo et al., 2012; Desjardins et al., 2015; Haag et al., 2014; Heinz et al., 2012; Katinka et al., 2001; Ndikumana et al., 2017; Pan et al., 2013; Pelin et al., 2016; 2015; Pombert et al., 2015; 2013; 2012; Reinke et al., 2017; Wiredu Boakye et al., 2017). The protein sequences were used to query the BUSCO microsporidian ortholog set using BLAST (Simao et al., 2015). Single copy orthologs shared in all 21 species were further used for

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Table 1
The list of 21 microsporidian species, strain, genome size, *Dicer* ortholog ID, *Argonaute* ortholog ID and isolated hosts. NA represents the ortholog was not found from the species.

species	strain	Genome size (Mbp)	Dicer ortholog ID	Argonaute ortholog ID	host
Anncaliia algerae	PRA339	12.1	KCZ79869.1	KCZ79938.1,KCZ79933.1	mosquito
Edhazardia aedis	USNM 41457	51.3	EJW03428.1	EJW02775.1, EJW02774.1	mosquito
Encephalitozoon cuniculi	EcunIII-L	2.2	NA	NA	lemming
Encephalitozoon hellem	ATCC 50504	2.2	NA	NA	rabbit
Encephalitozoon intestinalis	ATCC 50506	2.2	NA	NA	rabbit
Encephalitozoon romaleae	SJ-2008	2.1	NA	NA	grasshopper
Enterocytozoon bieneusi	H348	3.8	NA	NA	human
Enterocytozoon hepatopenaei	TH1	3.2	NA	NA	shrimp
Enterospora canceri	GB1	3.0	NA	NA	crab
Mitosporidium daphniae	UGP3	5.6	XP_013238173.1	XP_013237927.1	*Daphnia magna*
Nematocida parisii	ERTm1	4.0	NA	NA	worm
Nematocida sp.	ERTm5	4.3	NA	NA	worm
Nosema apis	BRL 01	8.5	EQB62224.1, EQB61196.1	EQB61131.1	honey bee
Nosema bombycis	CQ1	15.6	EOB15391.1	EOB14115.1	silkworm
Nosema ceranae	PA08	5.6	KKO76665.1	KKO75882.1	honey bee
Ordospora colligata	Oc4	2.2	NA	NA	*Daphnia magna*
Pseudoloma neurophilia	MK1	5.2	KRH94138.1	KRH92774.1, KRH92105.1	zebrafish
Spraguea lophii	42_110	5.7	EPR78224.1	EPR79492.1	monkfish
Trachipleistophora hominis		8.4	ELQ74212.1	ELQ74342.1	human
Vavraia culicis	floridensis	6.1	XP_008074153.1	XP_008074279.1	mosquito
Vittaforma corneae	ATCC 50505	3.2	XP_007605395.1	XP_007604198.1	human

phylogenetic analysis. Protein sequences of each of these orthologous groups (OGs) were aligned using Muscle with default settings (Edgar, 2004). Alignments were quality trimmed with trimAI (−w 3 −gt 0.95 −st 0.01) and then concatenated for phylogenetic analysis with Mrbayes (nchains = 4, aamodelpr = mixed, ngen = 1,000,000) (Capella-Gutiérrez et al., 2009; Ronquist and Huelsenbeck, 2003). The *Dicer* and *Argonaute* orthologs were retrieved from MicrosporidiaDB and OrthoDB databases and aligned using Muscle with default settings (Edgar, 2004), without trimming. Phylogeny trees were built with Mrbayes (nchains = 4, aamodelpr = mixed, ngen = 1,000,000) (Ronquist and Huelsenbeck, 2003). The trees were viewed with FigTree (http://tree.bio.ed.ac.uk/software/figtree/). *Mitosporidium daphniae* and *Botrytis cinerea* were used as out-group to root the species tree and gene tree respectively. A 2 by 2 contingency table was used to test whether *Dicer* and *Argonaut* were selected as a unit, as well as the impact of the host on the maintenance of *Dicer* and *Argonaut* orthologs. The raw and aligned sequences are provided in supplementary material.

3. Results

Of the 518 microsporidian single copy core genes, 18 ortholog groups were shared in all 21 parasite species. A phylogenetic tree of 21 parasite species was build based on the 18 ortholog groups (Fig. 1). From the 21 queried parasite species, eleven species maintained both *Dicer* and *Argonaute* orthologs (Table 1). The 11 parasite species maintaining *Dicer* and *Argonaute* orthologs were highlighted with color (Fig. 1). We found the parasites maintained both *Dicer* and *Argonaut* orthologs or lost both two orthologs, which was significantly different from random gene selection (Pearson's Chi-Square test, $P < 0.01$). Two clades were consistently found among species and gene trees. The clade I included four parasite species, *V. Culicis*, *T. hominis*, *P. neurophilia*, *S. lophii* and the clade II included *N. apis*, *N. ceranae* and *N. Bombycis* (Fig. 1). The same two clades were found in the phylogenetic tree of *Dicer* and *Argonaute* orthologs (Fig. 2), which was significantly deviated from random (Pearson's Chi-Square test, $P < 0.01$). *V. corneae* was closely related with clade I in the phylogenetic tree of *Argonaute* orthologs, but *V. corneae* was closely related with clade II in the phylogenetic tree of *Dicer* orthologs tree. In the species tree, *V. corneae* was not closely relates with neither clade I nor clade II.

To further study the presence of multiple orthologs of these genes, the protein sequences were inquired to the Pfam database. The domains were conservative between *N. apis* EQB61196.1 and EQB62224.1 (*Dicer* dimerization domain), as well as between *A. algerae* KCZ79933.1 and KCZ79938.1 (Piwi and PAZ domains). The two orthologs of the two species were cluster into the sister groups respectively (Fig. 2). However, *P. neurophilia*, KRH92105.1 and KRH92774.1 showed different domain predictions. The 60s Acidic ribosomal protein was predicted from KRH92105.1 and Piwi domain was predicted for KRH92774.1. The sequence of KRH92105.1 seems only partial from the gene prediction and, KRH92105.1 showed an earlier divergence compared with KRH92774.1 (Fig. 2B). The impact of hosts on the maintenance/loss of RNAi gene orthologs was further tested. A significant impact was not found neither between invertebrate and vertebrate, nor between insects and non-insects (Pearson's Chi-Square test, $P > 0.05$).

4. Discussion

In microsporidian parasites, two infection strategies have so far been found. First, the infections suppress the apoptosis of the infected cell to allow the parasite to complete at least one reproduction cycle (del Aguila et al., 2006; He et al., 2015; Higes et al., 2013; Martín-Hernández et al., 2017b). Secondly, the parasites secrete the hexokinase into the cytoplasm to accelerate the metabolism of the infected cells, which was conserved with a signal peptide (Cuomo et al., 2012). Additional gene knockdown studies found the ATP transporters, cell wall proteins, polar tube proteins are essential for the spore proliferation (Han et al., 2017; Li et al., 2016; 2012; Paldi et al., 2010; Xu and Weiss, 2005). Recently, microRNAs were identified from the microsporidian parasite *N. ceranae*, which potentially regulate over 10% host protein coding genes (Evans and Huang, 2018; Huang and Evans, 2016). By suppressing the gene expression of *Dicer*, the offspring spore load was significantly reduced (Huang et al., 2016a). The number and complexity of infection strategies adopted by the parasite may have impact on the genome size. Indeed, the species maintained RNAi orthologs also maintained transposable elements, which showed a larger genome sizes when compared with the ones lost them (Heinz et al., 2012; Ndikumana et al., 2017).

The Microsporidian genomes either maintained or lost both *Dicer* and *Argonaute* orthologs, which suggests that *Dicer* and *Argonaute* were selected as a unit. Within the 11 microsporidian species maintained RNAi genes, only 3 species showed multiple *Dicer* and *Argonaute* ortholog copies.*N. apis* showed two *Dicer* orthologs, which shared the same domains. *A. algerae* showed two *Argonaute* orthologs, which also shared the same domains. Considering the conserved protein domains

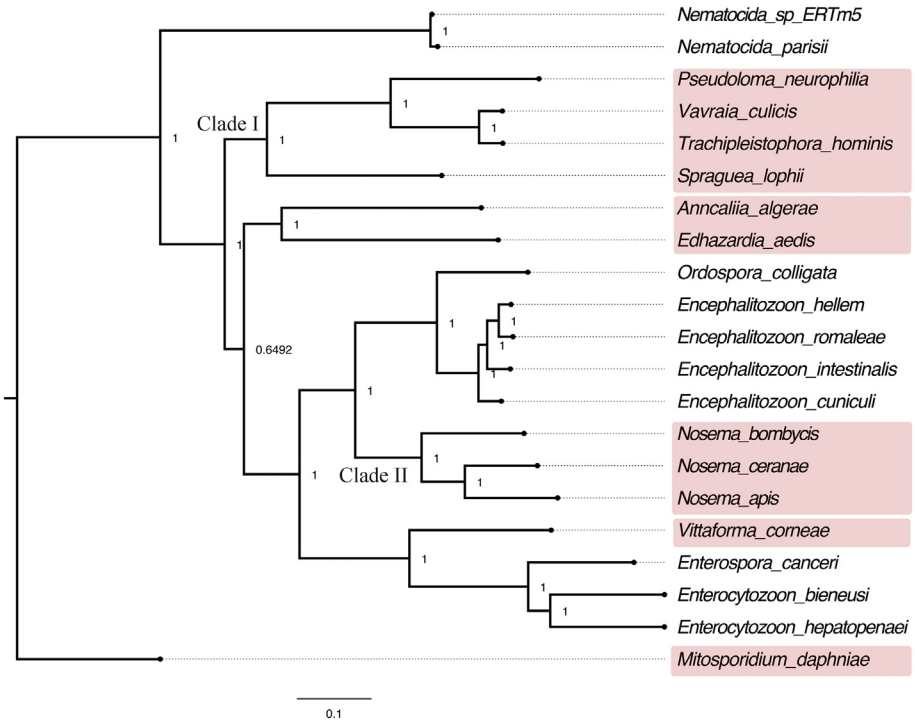

Fig. 1. Phylogenetic tree of 21 microsporidian parasite species. The rooted phylogenetic tree was constructed on protein sequences of 18 single copy orthologs shared among all parasite species. The 11 species with *Dicer* and *Argonaute* orthologs were marked with color. Out of 11 species, two clades were found between the species tree and the two gene trees. The clade I included four parasite species, *V. Culicis*, *T. hominis*, *P. neurophilia*, *S. lophii* and the clade II included *N. apis*, *N. ceranae* and *N. Bombycis*. The tree was constructed based on the maximum clade credibility with Mrbayes. Bayesian posterior probabilities were indicated at the nodes. The Legend for the branch length is shown at the bottom.

and short branch length between the orthologs, the multiple copies might due to recent duplication. *P. neurophilia* also showed two *Argonaute* orthologs. However, the two orthologs showed different protein domains and divergence. These two orthologs might have been placed different selective pressure during the lineage divergence. From another well studied fungus parasite, *B. cinerea*, two *Dicer* orthologs and five *Argonaute* orthologs were annotated, yet only one ortholog is essential to maintain a functional RNAi pathway (Tauati et al., 2014).

Overall the species within clade I and clade II were consistent between species tree and gene trees, which suggests the selection of RNAi genes occurs during genus/family or even higher-level lineage divergence. Even though the hosts' impacts on the selection of RNAi genes was not statistically significant in this study, I cannot conclude that the hosts are irrelevant with the selection of RNAi genes. This study provides preliminary insights to understand the selection of two RNAi gene orthologs and variance on the infection strategies for microsporidian parasitism during the evolution.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2018.08.011.

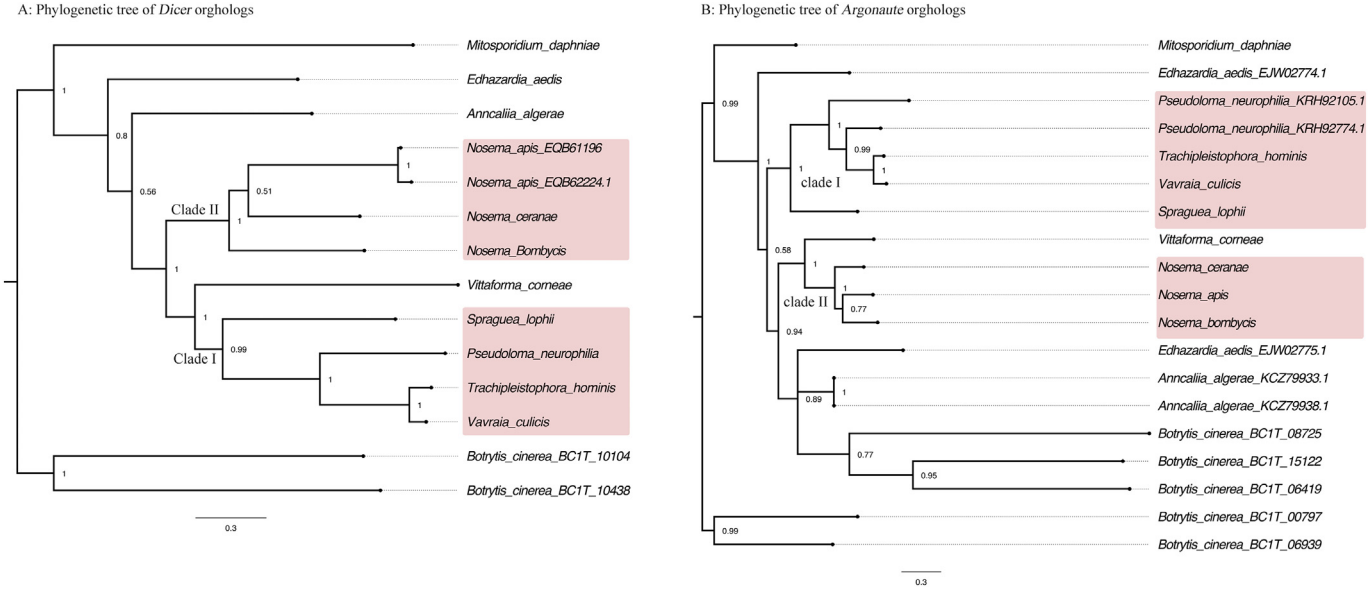

Fig. 2. Phylogenetic trees of *Dicer* and *Argonaute* orthologs. A: Rooted phylogenetic tree of *Dicer* orthologs based on 11 microsporidian parasite species with annotated *Dicer* orthologs. B: Rooted phylogenetic tree of *Argonaute* orthologs based on 11 microsporidian parasite species with annotated *Argonaute* orthologs. All the parasite species with annotated *Dicer* orthologs, also maintained *Argonaute* orthologs. The phylogenetic tree was constructed on protein sequences of *Dicer* and *Argonaute* orthologs. When multiple orthologs were found from one species, the protein sequence name was added to the species name. The trees were constructed based on the maximum clade credibility with Mrbayes. Bayesian posterior probabilities were indicated at the nodes. The Legend for the branch length is shown at the bottom.

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Conflict of interest statement

The author declares no conflict of interest.

Appendix A. Supplementary data

The raw sequences and alignments of BUSCO, *Dicer* and *Argonaute* orthologs. Supplementary data to this article can be found online at doi: https://doi.org/10.1016/j.meegid.2018.08.011.

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