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Molecular Characterization of Wenyonella philiplevinei from Ducks in Hunan Province, China

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Short Communication

Molecular Characterization of *Wenyonella philiplevinei* from Ducks in Hunan Province, China

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Abstract

Wenyonella philiplevinei is one of the important pathogens of duck coccidiasis. In the present study, a portion of the 18S rDNA was amplified from *W. philiplevinei* by Polymerase chain reaction (PCR), cloned and then sequenced. The sequences of 18S rDNA of all samples were 422 bp in size. A + T content of the 18S rDNA sequences was 58-59%. Sequence comparison revealed that the similarity in 18S rDNA sequences between Hunan isolates and *W. philiplevinei* available (Guangdong isolate) was more than 98%. Intra-specific sequence variations within each of the Hunan isolates were 0-1.7%. However, the inter-specific sequence differences between *W. philiplevinei* and coccidian available in GenBank were more than 7%. Phylogenetic analysis using neighbour joining (NJ), maximum likelihood (ML) and maximum parsimony (MP) methods indicated that the genus *Wenyonella* was more closely related to *Eimeria* + *Cyclospora* than to *Isospora*. These novel data provide useful genetic marker for the differentiation of *W. philiplevinei* or other closely related coccidians.

Keywords: 18S rDNA, duck, phylogenetic analysis, *Wenyonella philiplevinei*

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บทคัดย่อ

ลักษณะทางชีวโมเลกุลของ *Wenyonella philiplevinei* จากเป็ดในมณฑลหูหนานประเทศจีน

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Wenyonella philiplevinei เป็นหนึ่งในเชื้อที่สำคัญที่ก่อโรคบิดในเป็ด การศึกษาครั้งนี้ ส่วนหนึ่งของ 18S rDNA ของ *W. philiplevinei* ถูกเพิ่มปริมาณโดยปฏิกิริยาลูกโซ่โพลิเมอร์เรส (PCR) จากนั้นทำการโคลนและวิเคราะห์ลำดับเบส ลำดับของ 18S rDNA ของทุกตัวอย่างมีขนาด 422 bp ปริมาณ A และ T ของ 18S rDNA มีค่าร้อยละ 58-59 การเปรียบเทียบลำดับเบสแสดงให้เห็นว่ามีความคล้ายคลึงกันในลำดับ 18S rDNA ระหว่างเชื้อที่แยกได้จากหูหนาน และ *W. philiplevinei* (จาก Guangdong) มากกว่าร้อยละ 98 ความแตกต่างที่จำเพาะในแต่ละสายพันธุ์ของเชื้อที่แยกจากหูหนานคิดเป็นร้อยละ 0-1.7 อย่างไรก็ตามความแตกต่างที่จำเพาะระหว่าง *W. philiplevinei* และ coccidian ที่มีข้อมูลใน GenBank มีมากกว่าร้อยละ 7 การวิเคราะห์สายวิวัฒนาการ ด้วยวิธี Neighbour Joining (NJ), Maximum Likelihood (ML) และ Maximum Parsimony (MP) ชี้ให้เห็นว่า สายพันธุ์ Wenyonella มีความสัมพันธ์อย่างใกล้ชิดกับ *Eimeria* และ *Cyclospora* มากกว่า *Isospora* ข้อมูลใหม่เหล่านี้มีประโยชน์ในแง่ของการใช้เป็นเครื่องหมายทางพันธุกรรมสำหรับหาความแตกต่างของ *W. philiplevinei* หรือเชื้อบิดอื่นๆ ที่เกี่ยวข้อง

คำสำคัญ: 18S rDNA เป็ด phylogenetic analysis *Wenyonella philiplevinei*

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Introduction

Coccidiosis in ducks can be caused by members of the genera *Eimeria*, *Isospora*, *Tyzzeria* and *Wenyonella,* and seriously impairs growth and feed utilization of infected ducks resulting in production losses (Edgar, 1987). Coccidian is highly prevalent in ducks and *W. philiplevinei* is considered as predominant species in China (Yin et al., 1983). Previous investigations (Huang et al., 2004) showed that the *W. philiplevinei* is highly prevalent (30-80%) in many provinces or regions of China, including Henan, Xinjiang, Shanghai, Fujian, Yunan, Jiangxi, Beijing, Jiangsu and Guangdong. Although *W. philiplevinei* is considered a significant pathogen in ducks in China, until now, there is only one report of molecular characterization of *W. philiplevinei* (Chen et al., 2010). Furthermore, *W. philiplevinei* is also well recognized as the significant protozoan pathogens affecting poultry industry throughout the world (Skírnisson, 1997). However, little is known about the prevalence of *W. philiplevinei* in duck in other countries and regions. *W. philiplevinei* can cause serious damage to the digestive tract of the host, resulting in decreased bodyweight gain and diarrhea, and can even lead to death (Yin et al., 1983). Due to its high mortality, reduced production and control costs (Tewari and Maharana, 2011), the prevention of duck coccidiosis poses a challenge for the poultry industry development. Moreover, to our knowledge, no information is available about phylogenetic relationships of *W. philiplevinei*. The objectives of the present study were to characterize 18S rDNA sequences of the *W. philiplevinei* in ducks from China. Moreover, based on the 18S rDNA sequences, the phylogenetic relationships in *W. philiplevinei* were also reconstructed.

Materials and Methods

Parasites and isolation of genomic DNA: Oocysts of *W. philiplevinei* were obtained from feces of ducks in Linwu county, Hunan Province, China. The oocysts of *W. philiplevinei* were washed in physiological saline, identified primarily based on morphological characters and predilection sites to species (Zhang et al., 1999), and then were stored in 2.5% potassium dichromate at 4^0C. Purified oocysts of the *W. philiplevinei* were washed in phosphate buffered saline (PBS), disrupted in glass beads, and the total genomic DNA was extracted from individual samples by sodium dodecyl sulphate/proteinase K treatment, column-purified (Wizard™ DNA Clean-Up, Promega) and eluted into 50 µl distilled water according to the manufacturer's recommendations.

Enzymatic amplification: A portion of the 18S rDNA was amplified with primers Q1 and Q2 (Chen et al., 2010). These primers were synthesied on a Biosearch Model 8700 DNA synthesizer (Shanghai, China). PCR reactions (25 µl) were performed in 2 mM of $MgCl_2$, 2.5 µM of each primer, 2.5 µl 10×rTaq buffer, 0.2 mM of each dNTPs, 1.25 U of rTaq DNA polymerase (Takara, Japan), and 2 µl of DNA sample in a thermocycler (BioRad, USA) under the following conditions: after an initial denaturation at 94^0C for 5 min, then 94^0C for 30 sec (denaturation); 48^0C for 30 sec (annealing); 72^0C for 1 min (extension) for 35 cycles, followed by a final extension at 72^0C for 10

min. Five microlitres of each amplicon was examined by 0.8% (w/v) agarose gel electrophoresis to validate amplification efficiency. PCR products were sent to Sangon Company (Shanghai, China) for sequencing for both directions.

***Sequences analysis and phylogenetic reconstruction*:** Sequences of the 18S rDNA were separately aligned using Clustal X 1.83 computer program (Thompson et al., 1997). The sequences of 18S rDNA available in this study were used for phylogenetic analyses. Three methods, namely neighbour joining (NJ), maximum likelihood (ML) and maximum parsimony (MP), were used for phylogenetic re-constructions. NJ analysis was carried out using Dayhoff matrix model implemented by MEGA 4.0 (Tamura et al., 2007). ML analysis was performed using PUZZLE 4.1 under default setting (Strimmer and Haeseler, 1996) and Standard unweighted MP was performed using package Phylip 3.67 (Felsenstein., 1995). Consensus tree was obtained after bootstrap analysis, with 100 for ML tree, and 1000 replications for NJ and MP trees, with values above 50% reported. To study the genetic relationships among Eimeriidae coccidians, other Eimeriidae coccidians were considered in the present study (*Eimeria adeneodei* AF324212; *Eimeria meleagrimitis* KC305200; *Eimeria trichosuri* FJ829323; *Eimeria scholtysecki* AF324216; *Isospora suis* ISU97523; *Isospora felis* ISRRGE; *Cyclospora colobi* AF111186; *Cyclospora papionis* AF111187), with *Cryptosporidium parvum* (GenBank accession no: AF108864) as the outgroup. Phylograms were drawn using the Tree View v. 1.65 program (Page, 1996).

Results and Discussion

In the present study, specimens of *W. philiplevinei* infecting ducks from China were characterized on the bases of sequences of 18S rDNA regions. Many previous studies have indicated that 18S rDNA sequences can provide useful genetic markers for accurate identification and differentiation of coccidian species (Wünschmann et al., 2010; Ogedengbe et al., 2011; Carlson-Bremer et al., 2012).

In the present study, genomic DNA was extracted from 10 individual *W. philiplevinei* in Hunan province, China. Sequences of the 18S rDNA (~ 430 bp) were amplified individually and subjected to agarose gel electrophoresis (Fig 1). The results indicated that no size mutation was detected on agarose gels among any of the amplicons examined, and no products were also amplified from host DNA.. The sequences of 18S rDNA were 424 bp in length. The A + T content of the 18S rDNA sequences was 58-59%. Sequence comparison revealed that the similarity in 18S rDNA sequences between Hunan isolates and the *W. philiplevinei* available (Guangdong isolate) were more than 98%. The intra-specific sequence variations within each of the Hunan isolates were 0-1.7% (Fig 2). However, the inter-specific sequence differences between *W. philiplevinei* and the coccidian available in GenBank were more than 7%. This result was consistent with that of a previous study (Chen et al., 2010).

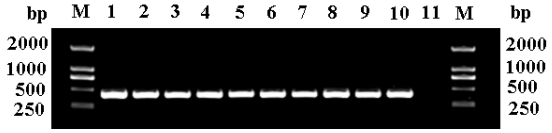

Figure 1 PCR-amplified results of 18S rDNA from *Wenyonella philiplevinei* samples by agarose gel electrophoresis. M: DL2000 DNA marker; 1-10: WP1-W10; 11: Negative control.

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Chen TTTCTGTCGTGGTCATCCGGCTCCGCTTTACAGTGGATACTTGGTTCGCCCTTGGCATTTTTCCGGTATCTTTCCCCACTTAATTGTGTGGGGTTTGATTTCCGGAACATTTACTTTGAGAAAAATAGAGTGTTTCAAGCAGGCTTGTCGC 150
WP1  ..............................................................................................................................................150
WP8  ..............................................................................................................................................150
WP10 ..............................................................................................................................................150
WP4  ..............................................................................................................................................150
WP3  ..........................................................T.....................T..............................................150
WP6  ..........................................................T.....................T..............................................150
WP7  ..........................................................T.....................T..............................................150
WP2  ..........................................................................TT.............................................150
WP5  ..........................................................................TT.............................................150
WP9  ..........................................................................TT.............................................150

Chen CTTGAATACTGCAGCATGGAATAATAAGATAGGACTTTAGTTCTATTTTGTTGGTTTCTAGGACTGAAGTAATGATTAATAGGGACAGTTGGGGGCATTCGTATTTAACTGTCAGAGGTGAAATTCTTAGATTTCTTAAAGACGAACTAC 300
WP1  ..............................................................................................................................................300
WP8  ..............................................................................................................................................300
WP10 ..............................................................................................................................................300
WP4  ..............................................................................................................................................300
WP3  ..............................................................................................................................T...............300
WP6  ..............................................................................................................................T...............300
WP7  ..............................................................................................................................T...............300
WP2  ..............................................................A...............................................................300
WP5  ..............................................................A...............................................................300
WP9  ..............................................................A...............................................................300

Chen TGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGACAGTAGGGGGTTTGAAGACGATTAGATACCGTCGTAATCTCTACCATAAACTATGCCGACTAGAGATAGGGAAACGCCT                                422
WP1  ..............................................................................................................                                422
WP8  ..............................................................................................................                                422
WP10 ..............................................................................................................                                422
WP4  ..............................................................................................................                                422
WP3  ..........................................................T.....................................................                                422
WP6  ..........................................................T.....................................................                                422
WP7  ..........................................................T.....................................................                                422
WP2  .......C..................................................................................................                                422
WP5  .......C..................................................................................................                                422
WP9  .......C..................................................................................................                                422
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Figure 2 Sequence comparison of 18S rDNA from *Wenyonella philiplevinei* in ducks.

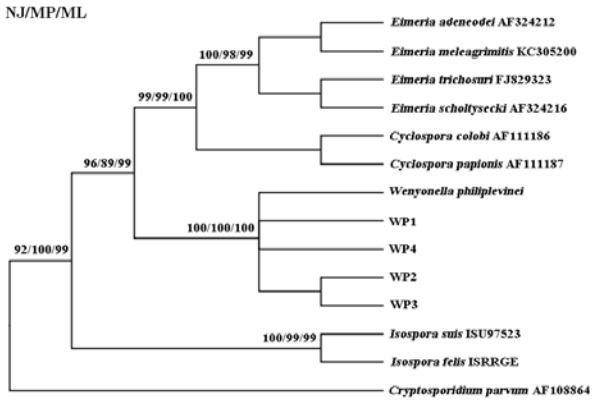

Figure 3 Phylogenetic relationship among the examined cestode species inferred by neighbour joining (NJ), maximum likelihood (ML) and maximum parsimony (MP) analyses based on 18S rDNA sequences, using *Cryptosporidium parvum* as outgroup.

Phylogenetic relationships among the examined *W. philiplevinei* were inferred based on 18S rDNA sequences. Topologies of all trees constructed by different methods (NJ, MP, and ML) with different distance models were similar, with only slight difference of bootstrap values (Fig 3). These results indicate that all the isolates in Hunan province represent *W. philiplevinei*. From the phylogenetic tree, genus *Eimeria* were sister to the *Cyclospora*, consistent with that of previous study (Lopez et al., 1999). The *Wenyonella* were more closely related to the *Eimeria* + *Cyclospora* than to the *Isospora*, with strong support in all of the three phylogenetic analyses (Fig 3).

In conclusion, the 18S rDNA sequences of the *W. philiplevinei* were characterized in ducks from China. The results of the present study have implications for the identification of *W. philiplevinei* infections in ducks in China and elsewhere.

Acknowledgements

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