

"Comments to the Author".

General comments:

The main objective of the manuscript is very interesting with promising results. The authors have worked hard but the manuscript needs be more attractive. The authors should propose which is the real utility of this study. I mean, with this discovery is possible, for example, to develop poly and monoclonal antibodies against to this new target for diagnosing or the treatment of the Pebrine.

Likewise, the authors should modify several expressions, concepts or correct form faults for a better understanding of the manuscript.

Abstract:

- IPTG: Enter the meaning of the acronyms.

- The authors should finish or conclude this epigraph with a possible utility after the description of this new target (*NpSWP9*). This contribution would be justifying the study carried out.

Introduction:

- Lines 18-19: I think is more appropriate, "ranged from invertebrate and vertebrate hosts (human, wild and domestic animals)".

- Lines 24-25: "More than 1500 species of microsporidia are found at present, about 14 genus can infect human": More than **1300** species of microsporidia are found at present, **17 species in 9 genera** can infect human.

(Deng, L., Li, W., Zhong, Z., Gong, C., Liu, X., Huang, X., et al. (2016). Molecular characterization 331 and multilocus genotypes of *Enterocytozoon bieneusi* among horses in southwestern China. 332 Parasit. Vectors. 9, 561. doi: 10.1186/s13071-016-1844-3.

- Line 25: "especially the species *Eenterocytozoon* and *Encyphalitozoon*": especially the **genera *Enterocytozoon* and *Encephalitozoon***.

- Line 26: "The immune system of a healthy people can exclude the microporida from body": I think is more appropriate "The human microsporidiosis can be asymptomatic or cause a self-limiting diarrhea, mainly in immunocompetent persons. However, it may cause persistent diarrhea, weight loss and malabsorption in immunocompromised patients".

- Line 28: "The genus Nosema is one": The genus ***Nosema*** is one.

- Line 29: "*Nosema bombycis*, parasitize in *Bombyx mori*": *Nosema bombycis*, **parasitizes to *Bombyx mori***.

- Line 33: "The main structure of the spherical microsporidia contains:": The main structure of the spherical microsporidia (**the spore**) contains.

- Line 34: “protoplasts, nucleus and polar filament”: **polaroplast**, nucleus, **posterior vacuole** and polar filament.
- Line 36: “thick exospore wall can help them defense the innate immunity”: thick exospore wall can help them **protect against to** the innate and **adaptive** immunity.
- Line 36: “or adverse period *in vitro*”. I don’t understand very well this expression. The authors should clarify the meaning.
- Line 38: “swallowed by the hosts”: **Phagocytosed** by the **host cell**.
- Line 42: “in the form process of spore wall”: in the form process of spore wall (**sporogony phase**).
- Line 44: “Ultrathin immunofluorescence microscopy”. A question: By Immunofluorescence-Antibody-Test (IFAT)?.
- Line 46: “genus Encephalitozoon”: genus **Encephalitozoon**.
- Line 49: “EhSWP1a of *E. cuniculi* and EnSWP1b of *Encephalitozoon hellem*”: **EhSWP1a and EhSWP1b** of *Encephalitozoon hellem*.
- Lines 50-51: “EnP1of *E. cunicul* and *E. intestinalis* respectively; EnP2 and SWP3 of *E. unculi*, EcCDA of *E. cuniculi* and *E. intestinalis*”: **EnP1 of *E. cuniculi* and *E. intestinalis***; EnP2 and SWP3 of ***E. cuniculi*, and EcCDA for *E. cuniculi* and *E. intestinalis***.
- Line 53: “Using boiling method and SDS method”: **In a study** using boiling method and SDS method.
- Line 59: “homologous to *N. bombycis* spore”: homologous to ***N. bombycis*** spore.
- Line 64: “for the further studies on the microsporidia”. The authors should propose, clarify or concrete something. For example, with this discovery is possible to develop poly and monoclonal antibodies against to this new target for diagnosing or the treatment of the Pebrine.

In my opinion with these proposals the authors would be justifying the study developed.

Materials and Methods:

- Line 68: “on a diet of fresh *Quercus liaotungensis* plantation”: on a diet of fresh ***Quercus liaotungensis*** plantation.
- Line 73: “*N. pernyi* were purified”: ***N. pernyi*** were purified.
- Line 77: “2.3 MORPHOLOGICAL OBSERVATION”: 2.3 MORPHOLOGICAL OBSERVATION BY TRANSMISSION ELECTRON MICROSCOPY (TEM).
- Line 81: “under a Transmission electron microscopy”: under a TEM.
- Line 87: “EST”. Enter the meaning of the acronyms.

- Line 90: "17.3 µl H₂O": 17.3 µl **H₂O**.
- Line 90: "2.5 µl 10×PCR buffer": 2.5 µl PCR buffer (**10×**).
- Line 90: "2 µl 5 mM dNTP": 2 µl 5 mM **dNTPs**.
- Line 91: "1 µl of each 10 mM primer": 1 µl of each primer (**10 mM**).
- Line 112: "transferred into BL21": transferred into BL21 (**competent *E. coli* cells**).
- Line 113: "adding IPTG (200 mg/ml)": adding IPTG (**inducer of protein expression to 200 mg/ml**).
- Line 114: "SDS-PAGE was performed observing the protein expression". The authors should include a reference of this methodology.

Results and Discussion:

- Line 123: "belonging to the Nosema Genus": belonging to the ***Nosema*** Genus.
- Line 127: "by transmission electron microscopy": by **TEM**.
- Lines 130-132: In this paragraph, I think, are the comments of the other reviewer or annotations of the authors. Anyway, I agree this opinion, and the results should be more clearly presented. Usually in this section the results are shown first and later the discussion is carried out.
- Line 139: "NpSWP9 from *N. pernyi*". The authors should unify if NpSWP9 is in italics. Review all the manuscript.
- Lines 142-143: "To assess the phylogenetic relationship between NpSWP9 and other microsporidia spore wall protein 9 or similar proteins". The phrase is unconnected. The authors should better connect it with previous sentence.
- Lines 145-146: "The result indicated that different spore wall proteins were assigned to corresponding groups related with different host". The authors should specify and indicate "with different host". List some of them in each case.
- Line 154-155: "they can infect most vertebrates and invertebrates": they can infect most **vertebrate and invertebrate hosts**.
- Line 161: "Anti-exospore antibody can reduce the microsporidia infection". The phrase is unconnected. The authors should better connect it with previous sentence.
- Lines 168-169: "SWP5 in *N. bombycis* is a spore wall localized": SWP5 in *N. bombycis* is a spore wall **protein** localized.
- Line 172: "Other two proteins, SWP7 and SWP9, both": Other two proteins **from *N. bombycis*, are SWP7 and SWP9, both**.

- In my opinion, the Discussion should finish with several proposals for the future with the discovery of this new target (*NpSWP9*). These proposals would help to justify all the study carried out by the authors.

Tables:

- Table 1: a) “location”: Location.

b) “Length ((bp)”: Length (**bp**)

c) “pI”: In the table foot, (pI: isoelectric point).

d) “reference”: Reference.

Figures:

- Figure 1: No comment.

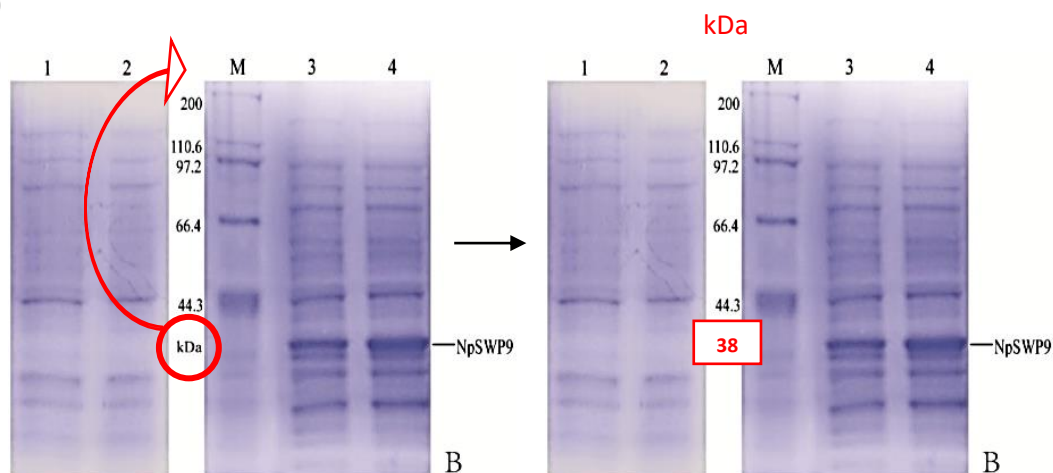
- Figure 2: No comment.

- Figure 3: No comment.

- Figure 4: “Fig. 4. Phylogenetic analysis of the *NpSWP9* from *N. pernyi* and other microsporidia by using TreeView (version 1.6.6)”: Phylogenetic analysis of the ***NpSWP9*** from *N. pernyi* and other microsporidia by using TreeView (version 1.6.6).

- Figure 5:

a)



b) “Fig. 5. Recombinant *NpSWP9-E1* Expression Vector (A) and SDS-PAGE detect *NpSWP9* protein expression (B)”: Fig. 5. Recombinant *NpSWP9-E1* Expression Vector (A) and SDS-PAGE detect ***NpSWP9*** protein expression (B).

References:

- Include this reference: Deng, L., Li, W., Zhong, Z., Gong, C., Liu, X., Huang, X., et al. (2016). Molecular characterization and multilocus genotypes of *Enterocytozoon bieneusi* among horses in southwestern China. Parasit. Vectors. 9, 561. doi: 10.1186/s13071-016-1844-3.

For the lines 24-25 (Introduction): More than **1300** species of microsporidia are found at present, **17 species in 9 genera** can infect human.