Balantidium grimi n. sp. (Ciliophora, Litostomatea), a new species inhabiting the rectum of the frog Quasipaa spinosa from Lishui, China

Weishan Zhao1,3, Can Li2, Dong Zhang1,3, Runqiu Wang1,3, Yingzhen Zheng4, Hong Zou1, Wenxiang Li1, Shangong Wu1, Guitang Wang1, and Ming Li1,∗

1 Key Laboratory of Aquaculture Disease Control, Ministry of Agriculture, and State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, 430072 Wuhan, PR China
2 Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, 430023 Wuhan, PR China
3 University of the Chinese Academy of Sciences, 100049 Beijing, PR China
4 Animal Husbandry and Aquaculture Station, Agriculture Forestry Animal Husbandry and Aquaculture Bureau of Guye District of Tangshan City, 063100 Tangshan, PR China

Received 27 January 2018, Accepted 1 May 2018, Published online 28 May 2018

Abstract — Balantidium grimi n. sp. is described from the rectum of the frog Quasipaa spinosa (Amphibia, Dicroglossidae) from Lishui, Zhejiang Province, China. The new species is described by both light microscopy (LM) and scanning electron microscopy (SEM), and a molecular phylogenetic analysis is also presented. This species has unique morphological features in that the body shape is somewhat flattened and the vestibulum is “V”-shaped, occupying nearly 3/8 to 4/7 of the body length. Only one contractile vacuole, situated at the posterior body, was observed. The phylogenetic analysis based on SSU-rDNA indicates that B. grimi groups together with B. duodeni and B. entozoon. In addition, the genus Balantidium is clearly polyphyletic.

Keywords: Balantidium grimi, ciliate, new species, Quasipaa spinosa, China

1 Introduction

The genus Balantidium Claparède & Lachmann, 1858 consists of many species inhabiting the digestive tract in a wide number of hosts from both invertebrate and vertebrate animals as endocommensals. They are generally considered harmless, but factors depressing the resistance of the host enable them to invade the mucosa and cause ulceration. The representatives of Balantidium have some common morphological features: cell body sacciform or slightly elongated in shape, and completely covered with cilia forming dense longitudinal rows [21]. To our knowledge, 31 amphibian balantidial species have been reported so far (lists in Li et al. [20]).

To date, 27 valid species have been reported in anuran amphibians, including B. amygdalli Bhatia & Gulati, 1927 [3], B. aurangabadensis Shete & Krishnamurthy, 1984 [34], B. bicavata Bhatia & Gulati, 1927 [3], B. claperedei Mahoon & Khan, 1986 [22], B. corlissi Shete & Krishnamurthy, 1984 [34], B. cyanophlycti Shete & Krishnamurthy, 1984 [34], B. duodeni Stein, 1867 [36], B. elongatum Stein, 1867 [36], B. entozoon Ehrenberg, 1838 [9], B. falciformis Walker, 1909 [40], B. ganapatii Shete & Krishnamurthy, 1984 [34], B. giganteum Bezzanger, 1904 [2], B. gracile Bezzanger, 1904 [2], B. helenae Bezzanger, 1904 [2], B. honghuensis Li et al., 2013 [18], B. kirbyi Rodriguez, 1939 [31], B. megastomae

*Corresponding author: liming@ihb.ac.cn.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Specimens were observed, measured and photographed using a microscope (Olympus BX53, Japan). All measurements are in micrometers.

2.3 Scanning electron microscopy

The fully washed specimens were fixed in 2.5% glutaraldehyde in 0.2 M PBS (pH 7.4) on a clean glass slide (1 cm × 1 cm), which was previously treated with 0.1% poly-L-Lysine and dried completely in the air at room temperature. After being washed with PBS 3 times, they were post-fixed in 1% osmium tetroxide at 4°C for 1 h, followed by serial dehydration in acetone and critical point drying using the HCP-2 critical point dryer (Hitachi Science Systems, Japan). Subsequently, the glass slide was mounted on an aluminum stub using a double-sided adhesive tape and sputter-coated with a thin layer of gold in IB-3 ion coater (Eiko Engineering, Japan), before observation and photography using a Quanta 200 SEM (FEI, Netherlands).

2.4 Extraction of genomic DNA and PCR amplification

About 50 individuals were harvested, suspended in lysis buffer (10 mM Tris-HCl, pH 8.0; 1 M EDTA, pH 8.0; 0.5 % sodium dodecyl sulfate; 60 µg/mL proteinase K), and incubated at 55°C for 12–20 h. DNA was extracted using a standard phenol/chloroform method, precipitated with ethanol, and resuspended in TE buffer. Polymerase chain reaction (PCR) amplifications were carried out using forward primer (5'-AACCTGGTGTGACCCCTGCA-3') and reverse primer (5'-TGATCCTTCTGT-CAGTTCACCTAC-3') [23]. The following cycling conditions included 5 min initial denaturation at 94°C; 35 cycles of 30 s at 95°C, 1 min at 56–60°C, and 1–2 min at 72°C; with a final extension of 10 min at 72°C. The PCR products were isolated using 1% agarose gel electrophoresis and purified using the Agarose Gel DNA Purification Kit (TaKaRa Biotechnology, Dalian, Japan). The amplified fragment was cloned into a pMD®18-T vector (TaKaRa Biotechnology, Dalian) and sequenced in both directions using M13 forward and reverse primers on an ABI PRISM® 3730 DNA Sequencer (Applied Biosystems, USA). The SSU rRNA gene sequence of B. grimi was deposited in GenBank with accession number MG837094.

2.5 Phylogenetic analysis

Besides the SSU-rDNA sequence of B. grimi that we obtained in this study, other litostomatean sequences were retrieved from the GenBank/EMBL databases (Table 1). The sequence of Nyctotheroides deslierresae was used as the outgroup. The secondary structure-based SSU-rRNA sequence alignment of Litostomatea downloaded from the SILVA ribosomal RNA gene database project (https://www.arb-silva.de/) [29] was used as the “seed” alignment to build a profile Hidden Markov Model (HMM) using HMMER Package, version 3.1. Then the HMM profile
| Species | GenBank/EMBL accession number | Reference |
|---------|-----------------------------|-----------|
| Trichostomatia Vestibuliferida | | |
| *Balantidium polyvacuolum* | KJ124724 | Li et al. [19] |
| *Balantidium ctenopharyngodoni* | GU480804 | Li et al. [19] |
| *Balantidium entozoon* | EU581716 | Grim and Buonanno [12] |
| *Balantidium duodeni* | KM057846 | Chistyakova et al. [7] |
| **Balantidioides grimi** | MG837094 | present study |
| *Balantidioides coli* (syn. *Balantidium coli*) | AM982723 | Ponce-Gordo et al. [27] |
| Isotricha intestinalis | U57770 | Wright and Lynn [41] |
| Isotricha prostoma | AF029762 | Strüder-Kypke et al. [38] |
| Lattea media | AB794981 | Ito et al. [14] |
| Lattea polyfaria | AB794982 | Ito et al. [14] |
| Parasotricha minuta | AB794984 | Ito et al. [14] |
| Parasotricha colpoidea | EF632075 | Strüder-Kypke et al. [37] |
| Buxtonella sulcata | AB794979 | Ito et al. [14] |
| Macropodiniida | | |
| Anglovorax dehorityi | AF298817 | Cameron et al. [4] |
| Anglovorax dogieli | AF298825 | Cameron et al. [4] |
| Bitricha tasmaniensis | AF298821 | Cameron et al. [4] |
| Bandia cribbi | AF298824 | Cameron and O’Donoghue [5] |
| Bandia deveneyi | AY380823 | Cameron and O’Donoghue [5] |
| Polycosta turniae | AF298818 | Cameron et al. (unpublished) |
| Macropodinium galabense | AF042486 | Wright (unpublished) |
| Macropodinium ennuensis | AF298820 | Cameron et al. [6] |
| Entodiniomorphida | | |
| Cycloposthium bipalmatum | AB530165 | Imai et al. (unpublished) |
| Troglodytelia abrassarti | AB437347 | Irbis et al. [13] |
| Ophryoscolex parkyngei | U57768 | Wright and Lynn [42] |
| Epidinium caudatum | U57763 | Wright and Lynn [42] |
| Entodinium caudatum | U57765 | Wright and Lynn [42] |
| Diplodinium dentatum | U57764 | Wright and Lynn [42] |
| Polyplastron multivesiculatum | U57767 | Wright et al. [43] |
| Eudiplodinium maggii | U57766 | Wright and Lynn [42] |
| Haptoaria | | |
| Dileptus sp. | AF029764 | Strüder-Kypke et al. [38] |
| Homalozoon vermiculare | L26447 | Leipe et al. [17] |
| Enchelys polynucleata | DQ411861 | Strüder-Kypke et al. [38] |
| Spathidium stammeri | DQ411862 | Strüder-Kypke et al. [38] |
| Didinium nasutum | U57771 | Wright and Lynn [41] |
| Pleurostomatida | | |
| Amphipleptus procerus* | AY102175 | Zhu et al. (unpublished) |
| Loxophyllum rostratum | DQ411864 | Strüder-Kypke et al. [38] |
| Armophorea | | |
| Clevelandellida | | |
| Nyctotheroides desliereae | AF145353 | Affa’a et al. [1] |

* submitted as *Hemiophrys procerus*, according to Strüder-Kypke et al. [37].
obtained was used to create an alignment of the 40 sequences using Hmmalgin within the package. The masked regions that could not be aligned unambiguously were removed from the initial alignment using MEGA 6.0 [39]. A GTR+I+G model was selected as the best model by the program jModelTest 2.1.10 [8] based on the AIC criterion, which was used for both Maximum Likelihood (ML) and Bayesian (BI) inference analysis. An ML tree obtained was used to create an alignment of the 40 sequences using Hmmalgin within the package. The masked regions that could not be aligned unambiguously were removed from the initial alignment using MEGA 6.0 [39]. A GTR+I+G model was selected as the best model by the program jModelTest 2.1.10 [8] based on the AIC criterion, which was used for both Maximum Likelihood (ML) and Bayesian (BI) inference analysis. An ML tree was constructed with the RaxML program [35]. The reliability of internal branches was assessed using the non-parametric bootstrap method with 1,000 pseudoreplicates. A Bayesian analysis performed with MrBayes v3.2.6 [32] was run for 1,000,000 generations sampling every 1,000 generations. All trees below the observed stationary level were discarded as a burn-in of 25% of the generations.

3 Results

Ninety-eight individuals of *Q. spinosa* were examined in the present study and 34 were found to be infected with *Balantidium grimi* (prevalence, 34.7%). These specimens were found mainly in the recta of frogs.

**Balantidium grimi** n. sp.
urn:lsid:zoobank.org:act:84E00073-0D0C-4166-8D83-20BFCC43480E

*Type host:* *Quasipaa spinosa* David, 1875.

*Prevalence:* 34.7% (34 of 98) of *Q. spinosa* were infected.

*Type locality:* Lishui City (27°25′–28°57′N, 118°41′–120°26′E), Zhejiang Province, China.

*Infection site:* Rectum.

*Type material:* Holotype catalogued under No. IHB2017W005, paratype catalogued under No. IHB2017W006 with protargol stained and the rest of ciliates preserved in 100% alcohol (Nos. LS001-002), 2.5% glutaraldehyde (No. LS003) and Bouin’s fluids (Nos. LS004-LS006) have been deposited in Key laboratory of Aquaculture Disease Control, Ministry of Agriculture, Institute of Hydrobiology, Chinese Academy of Sciences, China.

**Etymology:** The new species was designated *Balantidium grimi* n. sp. in honor of the great contributions of Prof. J. Norman Grim to parasitic and symbiotic ciliates.

3.1 Morphology under light microscope

Organism long-oval in shape (Figures 1A, C and 2), measuring 79.6-121.5 μm (X = 96.5 μm; n = 30) in length and 43.6-83.6 μm (X = 57.8 μm) in width. Body partially flattened and thickly ciliated (Figures 1A, C and 2). The number of body kineties ranged from 93 to 125, oriented mostly parallel to the cell’s long axis. Of these, 41 to 59 were dorsal and 52 to 67 were ventral. Vestibulum “V”-shaped, 32.6-53.9 μm (X = 43.43 μm, n = 30) in length, accounted for 3/8 to 4/7 of the body length (Figures 1B, D, E and 2), and 3.9-5.9 μm (X = 4.7 μm, n = 30) in width. Macronucleus oval and lay obliquely almost near the middle of body (Figures 1C, E, F and 2), 20.0-29.2 μm (X = 24.1 μm, n = 30) in length, and 12.4-19.3 μm (X = 16.0 μm, n = 30) in width. Micronucleus spherical or somewhat oval near the macronucleus (Figures 1C, E, F and 2), measuring approximately 2.2-2.9 μm (X = 2.5 μm, n = 13) in diameter. A distinct contractile vacuole situated at the posterior region of the body with 12.4-15.4 μm (X = 13.7 μm, n = 8) in diameter (Figures 1A and 2). A cytopyost present at the posterior end of the body (Figures 1A and 2). Detailed morphometric parameters are presented in Table 2.

3.2 Morphology under scanning electron microscope

*B. grimi* is thickly ciliated, but with uniform arrangement on the cell surface (Figures 3A, B). Regular beat patterns of cilia that look like “waves” make the cell move smoothly (Figure 3A). The “waves” and ridges

---

**Table 2.** Morphometric light microscopic parameters of *B. grimi.*

| Character                | X    | M    | Min  | Max  | SD   | SE   | CV(%) | N  |
|-------------------------|------|------|------|------|------|------|-------|----|
| Body length (Lb)        | 96.5 | 95.1 | 79.6 | 121.5| 9.65 | 1.76 | 10.0  | 30 |
| Body width              | 57.8 | 55.4 | 43.6 | 83.6 | 9.43 | 1.72 | 16.3  | 30 |
| Vestibulum length (Lv)  | 43.4 | 44.0 | 32.6 | 53.9 | 4.43 | 0.81 | 10.2  | 30 |
| Vestibulum width        | 4.7  | 4.7  | 3.9  | 5.9  | 0.44 | 0.08 | 9.4   | 30 |
| Macronucleus length     | 24.1 | 24.4 | 20.0 | 29.2 | 2.11 | 0.38 | 8.8   | 30 |
| Macronucleus width      | 16.0 | 16.1 | 12.4 | 19.3 | 1.88 | 0.34 | 11.8  | 30 |
| Micronucleus diameter   | 2.5  | 2.5  | 2.2  | 2.9  | 0.21 | 0.06 | 8.1   | 13 |
| Contractile vacuole diameter | 13.7 | 13.5 | 12.4 | 15.4 | 1.08 | 0.38 | 7.9   | 8  |
| Lb/Lv                   | 2.2  | 2.3  | 1.7  | 2.7  | 0.23 | 0.04 | 10.5  | 30 |
| Number of kineties on the left | 51.2 | 51   | 41   | 59   | 5.56 | 1.85 | 10.9  | 9  |
| Number of kineties on the right | 61.1 | 62   | 52   | 67   | 5.21 | 1.74 | 8.5   | 9  |

Measurements are in μm. X: arithmetic mean, M: median, Min: minimum, Max: maximum, SD: standard deviation, SE: standard error, CV: coefficient of variation, N: number of individuals investigated.
Figure 1. LM images of B. grimi. A. Specimens fixed in formalin (5%) and soaked in glycerine-alcohol (10%), showing the oval body shape, vestibulum (vb) and macronucleus (ma), a round contractile vacuole (cv) in the posterior and a cytoproct (cp) at the end of the body. Scale bar = 10 μm. B. Specimens fixed in formalin (5%) and soaked in glycerine-alcohol (10%), showing the long vestibulum (vb) surrounded by cilia. Scale bar = 10 μm. C-F. are protargol stained: C. showing the body shape, macronucleus (ma) and micronucleus (mi). Scale bar = 10 μm. D. showing the vestibulum and somatic kineties. Scale bar = 10 μm. E. showing the vestibulum (vb) and the oval macronucleus (ma) with a spherical micronucleus (mi) embedded in the middle. Scale bar = 10 μm. F. showing the relative position of macronucleus (ma) and micronucleus (mi). Scale bar = 5 μm.
formed an angle ranging from 0° (at the posterior) to 60° (at the anterior) (Figures 3A, C, D). Numerous cortical grooves arranged alternately with cortical ridges, which are parallel to the longitudinal axis of the body (Figure 3D). The cilia originate within grooves and are quite close together; those in Figure 3D are about 0.62 μm apart.

3.3 Phylogenetic analysis

The sequenced SSU-rRNA gene of *B. grimi* is 1,640 bases in length and the guanine-cytosine (GC) content is 42.26%. The topologies of our phylogenetic trees generated using MrBayes and PhyML algorithms are totally accordant (Figure 4). Species of the family Balantidiidae are separated into three clades. *B. grimi* grouped together with *B. duodeni* and the type species of the genus, *B. entozoon*, and form the first clade whose hosts are anuran amphibians (100% ML, 1.00 BI). *B. polyvacuolum* and *B. ctenopharyngodoni* form the second balantidial clade inhabiting fish hosts. The third group consisted of two isolates of *B. coli*, which were reported from many mammalian hosts, including pigs and humans.

4 Discussion

A new *Balantidium* species inhabiting Chinese anuran amphibians *Quasipaa spinosa* is recorded herein. To our knowledge, this is the first report of *Balantidium* species in *Q. spinosa*.

*B. grimi* is quite unique considering its remarkably flattened body and conspicuous slit-shaped vestibulum, which can distinguish it from other *Balantidium* species [7,12,21]. *B. grimi* resembles *B. entozoon*, *B. duodeni*, *B. helenae* and *B. sinensis* in some aspects. For example, *B. grimi* shares a similar Lv/Lb value with *B. duodeni* [7]. But in terms of body forms and dimensions, these two balantidial species could easily be discriminated from each other. As to the shape and dimension of the macronucleus, as well as the position of the contractile vacuole, *B. grimi* somewhat resembles *B. helenae* [33], but the latter species possesses a remarkable “knob” at the posterior end. Comparisons were also made between *B. grimi* and *B. sinensis* inhabiting the Chinese giant salamander *Andrias davidianus* [20] as well as *B. entozoon*, the type species of the genus *Balantidium* [12]. Detailed comparisons of morphometric parameters among corresponding *Balantidium* species are presented in Table 3.

According to the molecular phylogenetic analysis, the order Macropodiniida ciliates is closely related to fish balantidia species [14,19]. The affinity implies that macropodiniids may have been the result of separate invasions of terrestrial hosts by ciliates initially associated with aquatic hosts [19]. Macropodiniids, previously called “Australian clade”, possess similar oral cavities to some vestubuliferids that are bordered by somatic kineties and analogous ultrastructure to the Isotrichidae [5,21,37,38]. Moreover, the strong molecular support of Macropodiniida assemblage as a sister clade to the Balantidiidae (fish balantidia) also gives us an indication that Macropodiniida ought to be incorporated into the order Vestibuliferida, which also coincides with the viewpoint of former studies [5,14,19].

Our results show that the genus *Balantidium* is clearly polyphyletic and all *Balantidium* species are separated into three distinct clades, according to host specificity: fish balantidia (*B. ctenopharyngodoni* and *B. polyvacuolum*), amphibian balantidia (*B. grimi*, *B. entozoon* and *B. duodeni*), and balantidia from warm-blooded vertebrates (*Balantiodes coli*) [7]. Pomajbíková et al. [26] has proposed a new genus *Neobalantidium* for the third group. However, it was recently suggested to reinstate the genus *Balantioides* as this taxon has been named for a long time [7]. Here, we accepted the generic name *Balantioides* to describe this group. As to the amphibian balantidia, our new species clustered with the other two species, *B. entozoon* and *B. duodeni* with maximum molecular supports. On this point, our results are consistent with
Figure 3. SEM images of *B. grimi*. **A.** Overview of the ventral-left side (oral side), showing the general form, vestibulum (arrow) and uniformly arranged cilia. Scale bar = 10 μm. **B.** Overview of the right side, showing the body surface is partially flattened and thickly ciliated. Scale bar = 10 μm. **C.** Ventral-left view of the "V"-shaped vestibulum (arrow). Scale bar = 5 μm. **D.** The left anterior area of ciliate, showing the vestibulum (vb), an interkinetal ridge (rd), the groove (gr) and the cilia (cl) extending from grooves and are close to one another. Scale bar = 5 μm. **E.** Selected enlargement of Figure 3D, showing a ridge (rd) between cilia. Scale bar = 2 μm.

Table 3. Comparison of body length (Lb), vestibulum length (Lv) and the ratio of vestibulum length and body length (Lv/Lb) between *B. grimi* and four *Balantidium* species.

| Species          | Host               | Body length (Lb) | Vestibulum length (Lv) | Lv/Lb |
|------------------|--------------------|------------------|------------------------|-------|
|                  |                    | X    | Min  | Max   | X    | Min  | Max   |       |
| *Balantidium entozoon* | *Rana esculenta*   | 83.3 | 60.0 | 129.0 | 27.7 | 20.0 | 34.0  | 0.33  |
| *Balantidium duodeni*    | *Rana temporaria*  | 128.6| 111.6| 156.9 | 56.3 | 44.2 | 76.7  | 0.44  |
| *Balantidium helenae*    | *Rana ridibunda*   | 88.9 | 62.5 | 112.5 | 33.2 | 25.0 | 50.0  | 0.37  |
| *Balantidium sinensis*   | *Andrias davidianus*| 138.3| 120.0| 158.4 | 47.0 | 40.8 | 52.8  | 0.34  |
| *Balantidium grimi*      | *Quasipaa spinosa* | 96.5 | 79.6 | 121.5 | 43.4 | 32.6 | 53.9  | 0.44  |

Measurements are in μm. X: arithmetic mean, Min: minimum, Max: maximum.
those of Chistyakova et al. [7], but differ from those of Li et al. [19]. We suspect that the key reason for this disagreement is the quantity of introduced species used for phylogenetic analysis. The greater the number of related species studied, the greater the accuracy of the resulting phylogeny. Thus, more molecular information on Balantidium species from fishes and amphibians as well as reptiles is needed to clarify their phylogenetic relationships.

Acknowledgments. Financial support for this study was provided by the National Natural Science Foundation of China (No. 31772429, 31471978), the Youth Innovation Promotion Association CAS (No. Y82Z01), and the Earmarked Fund for China Agriculture Research System (No. CARS-45-15).

Conflict of interest

The authors declare that they have no competing interests.

Figure 4. Phylogenetic relationships of the SSU-rRNA sequences of B. grimi marked in bold and other Trichostomatia species showing the position of B. grimi inferred by maximum likelihood method and Bayesian algorithm. The trees were rooted using the sequence of Nyctotheroides deslierresae as the outgroup taxa. Numbers at nodes indicate bootstrap percentage and posterior probability, respectively. The sequences corresponding to species of the genus Balantidium are shadowed.

References

1. Affa’a FL, Hickey DA, Strüder-Kypke M, Lynn DH. 2004. Phylogenetic position of species in the genera Anoplophrya, Plagiotaema, and Nyctotheroides (Phylum Ciliophora), endosymbiotic ciliates of annelids and anurans. Journal of Eukaryotic Microbiology, 51(3), 310-316.
2. Bezzuenger E. 1904. Über Infusorien aus asiatischen Anuren. Archiv für Protistenkunde, 3, 138-174.
3. Bhathia BL, Gulati AN. 1927. On some parasitic ciliates from Indian frogs, toads, earthworms and cockroaches. Archiv für Protistenkunde, 57, 85-120.
4. Cameron SL, Adlard RD, O'Donoghue PJ. 2001. Evidence for an independent radiation of endosymbiotic litostome ciliates within Australian marsupial herbivores. Molecular Phylogenetics and Evolution, 20(2), 302-310.
5. Cameron SL, O'Donoghue PJ. 2004. Phylogeny and biogeography of the “Australian” trichostomes (Ciliophora: Litostomata). Protist, 155(2), 215-235.
6. Cameron SL, Wright A-DG, O’Donoghue PJ. 2003. An expanded phylogeny of the Entodiniomorphida (Ciliophora: Litostomatea). Acta Protozoologica, 42(1), 1-6.
42. Wright ADG, Lynn DH. 1997. Phylogenetic analysis of the rumen ciliate family Ophryoscolecidae based on 18S ribosomal RNA sequences, with new sequences from Diplodi- nium, Eudiplodinium, and Ophryoscolex. Canadian Journal of Zoology, 75(6), 963-970.

43. Wright ADG, Dehority BA, Lynn DH. 1997. Phylogeny of the rumen ciliates Entodinium, Epidinium and Polyplastron (Litostomatea: Entodiniomorphida) inferred from small subunit ribosomal RNA sequences. Journal of Eukaryotic Microbiology, 44(1), 61-67.

Cite this article as: Zhao W, Li C, Zhang D, Wang R, Zheng Y, Zou H, Li W, Wu S, Wang G, Li M. 2018. Balantidium grimi n. sp. (Ciliophora, Litostomatea), a new species inhabiting the rectum of the frog Quasipaa spinosa from Lishui, China. Parasite 25, 29