# 博士論文審査報告書

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| 氏名 | 梁瀬　隆二 |
| 学位の種類 | 博士（理学） |
| 学位記番号 | 博理第１０９号 |
| 学位授与報告番号 | 甲第３２７号 |
| 学位授与年月日 | 平成３０年３月２２日 |
| 学位授与の要件 | 学位規則第４条１項該当 |
| 論文題目 | The extension-contraction mechanism of the neck of a ciliate, *Lacrymaria olor*<br>「繊毛虫 *Lacrymaria olor* の首部の伸縮機構」 |
| 論文審査委員 | （主査）教授　宮澤　淳夫<br>（副査）教授　吉久　徹<br>（副査）教授　八田　公平<br>（副査）准教授　洲崎　敏伸<br>（神戸大学大学院理学研究科）<br>（副査）Keith Gull<br>(Professor, Sir William Dunn School of Pathology,University of Oxford, Oxford, UK)<br>（副査）Jack Sunter<br>(David Fell Research Fellow, Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK)<br>（副査）准教授　園部　誠司 |

※Gull 委員および Sunter 委員の審査結果については別紙（英文）として添付する。

１．論文内容の要旨

*Lacrymaria olor*（ロクロクビムシ）はリトストマ綱に属する自由生活繊毛虫で、その体制は細胞体、首部、口部の３つに分けられる。捕食中、*L. olor* は首部を様々な方向への急速な伸縮運動を行う。こうした高速の伸縮運動はこの生物の際立つ特徴のひとつである。これまでに首部の伸長が口部の繊毛によるものであるとの推測がなされており、また、微細構造学的研究によって微小管の存在が報告されているが、非常な高速運動のため解析が困難で、この運動の詳細なダイナミクスは明らかにされていなかった。申請者は高速度カメラを用いた画像解析を行い、４つの細胞状態、すなわち、active、resting、 activation、inactivation が存在することを明らかにした。また、高速度カメラを用いた画像解析から口部の繊毛運動が発生する力が首部の伸長に主として寄与していることを明らかにした。さ

らに微小ビーズを首部に付着させることによりその物理的性質を解析し、伸長時には非線型の粘弾性を持つこと、すなわち、先端部に向かって柔らかくなっていることを見出した。収縮時には首部全体が均一な収縮を示し、線型弾性体の性質を持つことを明らかにした。このことから、首部の収縮には何らかの収縮性因子が関与していることが予想された。このように本論文では *L. olor* 首部の伸縮運動の特徴を明らかにし、４つの細胞状態の存在を明らかにした。また、首部の伸長において口部繊毛が主体的な役割を果たしていることを明らかにした。さらに、首部の物理的性質を明らかにした。これらの結果を踏まえて *L. olor* の首部の伸縮運動の機構について議論した。

２． 論文審査結果

*L. olor* は捕食の際、首部の急速な伸長収縮という非常にユニークな運動形態を示す。申請者は高時間分解能の画像解析と物理的解析によりこの運動の機構を明らかにしようとした。その結果、i) 既知の２つの細胞状態に加え、これまで報告されていなかった'activation'と'inactivation'というもう２つの細胞状態の存在を明らかにし、ii) 首部の伸長に口部繊毛が主体的にかかわっていることを示し、iii) 伸長時と収縮時には異なった物理的性質を持ち、それぞれ異なった因子が関与していることを示した。これらの結果は、これまであいまいであった *L. olor* の運動の特徴を定量的に明らかにした点で高く評価できる。またこの成果は、次の研究段階への重要な手がかりを与えている。さらにこの研究は *L. olor* の首部の伸縮機構の研究に多大な貢献をしただけではなく、他の多くの原生生物のユニークな運動機構の解明に大きなインパクトを与えるものである。

よって、本論文は博士（理学）の学位論文として価値のあるものと認める。

また、平成３０年１月２３日、論文内容及び関連する事項について試問を行った結果、合格と判定した。

# Evaluation Report for Doctoral Thesis

Title：The extension–contraction mechanism of the neck of a ciliate, *Lacrymaria olor*

Applicant：Ryuji Yanase

## 1. Abstract of the thesis

*Lacrymaria olor* is a free-living ciliate protist belonging to a highly diverse class, Litostomatea. An *L. olor* cell consists of three parts, a cell body, an extendable neck and an oral apparatus on the distal end of the neck. During predation, *L. olor* rapidly extends its neck in various directions, and then the neck is contracted. Such a rapid extension–contraction cycle is one of the unique characters of *L. olor*. Although previous papers proposed that the extension of the neck was induced by the beating of oral cilia and clarified ultrastructural features including microtubules, dynamic features of the extension–contraction cycle of the neck in *L. olor* have been unclear because of experimental difficulty derived from rapid deformation of the neck. As the first step to elucidate the mechanism of such dynamic processes, the applicant characterized the deformation of the neck of living *L. olor* using a high-speed camera and image processing and succeeded in defining the four different cell states, namely, active, resting, activation and inactivation, and highlighted the features of the neck deformation of *L. olor* depending upon the cell states. Then, he conducted detailed analyses of the ciliary movement with a high-speed camera and showed that the oral ciliary movement mainly contributes to the extension power of the neck, and a contractile tension constantly exists in the neck even during its extension. In addition, high-speed imaging of the movement of the fluorescent microspheres attached to the cell surface revealed that changes of microsphere distance on the part of the neck near the tip were larger than those on the part of the neck near the cell body, suggesting that the neck has a property like a nonlinear viscoelastic body that gradually becomes softer toward the distal end during extension. On the other hand, changes of distance between microspheres during contraction indicated that each distance between two adjacent microspheres on the neck reduced almost at the same speed, suggesting that the neck has a property like a linear spring rather than nonlinear spring during contracting periods, and some contractile factors that work only during contracting periods exist in the neck. Thus, his findings shed light on the features of the neck deformation of *L. olor* and defined the cell states of *L. olor*. Also, he confirmed contribution of the oral ciliary movement for the neck extension and revealed characteristic physical properties of the neck. From these findings, he discussed the extension–contraction mechanism of the neck of *L. olor*.

## 2. Evaluation of the thesis and the final examination

A rapid extension–contraction cycle during predation is one of the unique characters of *L. olor*. In this thesis, the applicant tried to elucidate the mechanism of this unique movement using image analysis with high temporal resolution and physical analysis. As a result, he succeeded in i) defining two novel cell states, namely 'activation' and 'inactivation' states, in addition to previously defined active and resting states, ii) confirming the major contribution

of the ciliary movement on the oral part in producing the motive force for neck extension, iii) elucidating characteristic physical properties of the neck, suggesting involvement of different factors in extension and contraction, respectively. These findings are valuable in respect to elucidate the feature of this movement quantitatively, which had been unclear. In addition, this work provides clues of targets for the next studies, and a new insight into studies on the mechanism of not only the neck movement of *L. olor* but also those observed in a variety of protists.

Thus, the review committee members listed below hereby state our full approval of the thesis completed by the applicant in partial fulfillment of the requirements for the degree of Doctor of Science in the Graduate School of Life Science.

The committee also certifies that the applicant passed the final oral examination on his thesis and related issues held on January 23 in 2018.

The chief examiner: Atsuo Miyazawa _____

The second readers: Tohru Yoshihisa _____

Kohei Hatta _____

Toshinobu Suzaki _____
(Associate Professor, Graduate School of Science, Kobe University)

Keith Gull _____
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and contraction, respectively. These findings are valuable in respect to elucidate the feature of this movement quantitatively, which had been unclear. In addition, this work provides clues of targets for the next studies, and a new insight into studies on the mechanism of not only the neck movement of *L. olor* but also those observed in a variety of protists.

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