

Atlas of Aquatic Viruses and Viral Diseases

水生病毒及

病毒病图鉴

张奇亚 桂建芳 著 Qi-Ya Zhang Jian-Fang Gui



水生病毒及病毒病图鉴 Atlas of Aquatic Viruses and Viral Diseases

张奇亚 桂建芳 著 Qi-Ya Zhang Jian-Fang Gui



北京

内容简介

水生病毒学研究的对象是感染水生生物或存在于水环境中的病毒。本书源自著者相关研究的积累,兼具专著与图籍属性。从逾万幅图中遴选了约800幅(图或图版),其中彩图约200幅。本书分为6个部分:水生病毒病症及水生病毒学技术、水生哺乳动物病毒、爬行动物病毒、两栖动物病毒、鱼虾类动物病毒及淡水浮游病毒,共分23章,以图示和简洁的中英文图注直观展示了水生生物病毒病症状、宿主组织及其细胞的病理变化、水生病毒的超微形态及基因组成和蛋白定位等。

本书是为相关学科的学生和研究者所著,可供从事病毒学、水生生物学和环境科学等相关学科教学与研究的大专院校及科研单位图书馆收藏。

图书在版编目 (CIP) 数据

水生病毒及病毒病图鉴=Atlas of Aquatic Viruses and Viral Diseases/ 张奇亚,桂建芳著.—北京:科学出版社,2012 ISBN 978-7-03-035643-7

I. ①水··· Ⅱ. ①张···②桂··· Ⅲ. ①水生生物学-病毒学-图集 Ⅳ. ①Q175-64

中国版本图书馆CIP数据核字(2012)第228427号

责任编辑:罗静付聪/责任校对:宋玲玲责任印制:钱玉芬/书籍设计:北京美光设计制版有限公司

科学出版社出版

北京东黄城根北街16号 邮政编码: 100717 http://www.sciencep.com

北京通州皇家印刷厂 印刷 北京美光设计制版有限公司 制版

科学出版社发行 各地新华书店经销

定价: 280.00元

(如有印装质量问题, 我社负责调换)

前言

与细胞相比病毒很微小,且必须依赖宿主细胞才能复制。由于这些特征,研究病毒需要特定的技术和设备。通常只能通过显微镜(如电子显微镜)才能观察到病毒。能实时目睹病毒的结构、生命活动、引起机体的变化及其与宿主细胞的相互作用,一直是众多学者们感兴趣并积极追寻的佳境。水生生物和水环境病毒的研究不仅有助于人类更好地认识病毒的感染、形态发生及传播机制,潜在的病毒病治疗良方,而且有益于评价与开发生物资源,了解病毒的起源及与宿主的相关性,认识病毒对淡海水生态系统及生物化学循环的重大影响。

水生病毒(aquatic virus)通常是指以水生生物为宿主的病毒,包括水生动物病毒、水生植物病毒和水生微生物病毒。近年,随着海水和淡水中大量浮游病毒被发现,广义的水生病毒也包括在水体中存活的各类浮游病毒。

水生病毒学(aquatic virology)是水生生物学与病毒学的交叉学科,也是一门年轻学科。该学科的形成始于20世纪50年代的鱼类病毒学。20世纪五六十年代,借助细胞培养及电镜技术,尤其是连续培养的鱼类细胞用于鱼类病毒病分离,使之成为鱼类病毒学研究的一个重要发展时期。

20世纪六七十年代,随着全球水产业的快速发展及其流行病害的频繁发生,多种致死性鱼类病毒病被鉴定。1988年,美国的Wolf K. 教授撰写出版了第一部鱼类病毒学专著《鱼类病毒与鱼类病毒病》(纽约,康奈尔大学出版社)。20年之后,我们出版了第一本《水生病毒学》教科书(北京,高等教育出版社,2008)。过去50年,水生病毒学虽有显著地发展,但仍明显滞后于医学病毒学。

在本书中,著者试图通过直接的微观图(含许多尚未发表过的图)及相关技术, 如电镜观察和生化分析技术,呈现水生病毒学的发展轨迹,尽可能以简质求厚重,取 明晰达隽永。每幅图都可作为水生病毒学研究的一个实例,将有助于了解病毒和其感



染的水生生物之间的关系。进而,本书还以其独到的触角和视野,将读者带入更深入和更广泛的水生病毒研究领域。

武汉大学病毒学系是我国建立的首个病毒学系。著者之一(张奇亚)自进入武汉大学病毒学系开始,就预感此生将与病毒学有不解之缘,而摄撰并出版水生病毒图鉴就更是梦寐萦怀的夙愿。其本科毕业后,被幸运地分配到中国科学院武汉病毒研究所工作,然而初涉科坛,不抵山巅不知天高,不临深涧不明水深,憾少有积累。

在大学同窗挚友金明洁博士、李先强博士和付向东博士的引荐下,年逾而立的张奇亚得以远赴大洋彼岸到美国求学取经。其先在美国俄亥俄医学院(Medical College of Ohio, MCO)Keith D. Garlid教授指导下获得硕士学位后,又来到加州大学圣迭戈校区(University of California, San Diege, UCSD)美国科学院院士Micheal G. Rosenfeld的实验室继续深入学习和研究分子生物学技术。

与此同时,另一位著者桂建芳也正与在UCSD执教的付向东教授一起从事RNA加工和细胞周期调控机制研究。圣迭戈美丽的海滨和迷人的风光也同时催开了我们的学术和生命之花。1994年,要学成报国的我们满怀憧憬,来到中国科学院水生生物研究所。这里浓郁的学术氛围给了我们从事水生病毒学研究的良好机遇。

一直深怀感激的是在水生病毒学研究刚刚举步之时,我们得到几位德高望重的战略 科学家的引导与支持:时任水生生物研究所所长,后又任中国科学院副院长、国家自然科 学基金委(NSFC)主任的陈宜瑜院士,为我们提供了开展鱼类病毒学研究的机会及启动 科研基金的资助;时任国家淡水生态与生物技术实验室(FEBL)主任、又任中国科学院 水生生物研究所所长和国家自然科学基金委副主任的朱作言院士,提供了国家重点实验室 开放基金的资助;中国科学院水生生物研究所名誉所长刘建康院士审阅并校正了我们投向 《科学通报》的第一篇水生病毒文稿;中国鱼类学会理事长曹文宣院士为我们的研究提出

了有益的指导和建议。

还要感谢导师沈蕴芬院士和王伟俊研究员,是他们悉心引导,激励学生春耕秋收,衔华佩实。更要感谢中国科学院水生生物研究所现任所长赵进东院士,感谢他麾下中国科学院知识创新工程重要方向项目(KSCX2-EW-Z-3)的支持,给水生病毒学纵深拓展带来新的契机。

特别感谢多年来志同道合的团队成员及同仁们,他们是: 袁秀平、李正秋、朱蓉、陈中元、江育林、李彦、刘荭、罗晓春、肖枫、谢简、盛慧英、阮红梅、曾首英、刘艳鸣、杜昌升、吕爱军、孙伟、苗大利、徐唯、黄晓红、陶建军、黄友华、金唯信(朝鲜博士后)、赵哲、周广舟、柯飞、高恶斌、桂朗、雷晓颖、何利波、朱若林、李顺、裴超、李三华、欧铜、高小蝉、王俊、项碧玉等。无论是人在旅途穿梭奔忙,还是身置实验室埋头苦干,大家心以积虑而起悟,学以渐博而相通,都在为水生病毒学发展夙兴夜寐、殚精竭虑,奉献着智慧与力量。

虽骐骥一跃,不能十步,但驽马十驾,功在不舍(引自《荀子·劝学》箴言)。著者 倾多年从事水生病毒学研究之力,博观约取、厚积薄发,集众思、撷精粹,终于从数以万 计的图中遴拔集成了这本图鉴。虽仍有诸多缺憾,亟待完善,却记录了水生病毒学这门新 交叉学科发展的一段不寻常历程,集结了众多学人对这门学科的贡献。我们期盼这个学科 有更快的发展。

在本书出版之际,我们还要感谢中国工程院院士、华中农业大学陈焕春教授,武汉大学李文鑫教授和清华大学陈应华教授,是他们向国家科学技术学术著作出版基金委员会推荐了本书;感谢科学出版社王静和罗静的精湛编辑;还要向在本书出版过程中为我们提供支持的每位专家和朋友致以深深地敬意与感谢!

非常感谢给我们以支持的多个基金: 国家科学技术学术著作出版基金(2010-c-



002);国家"973"计划(G1999053908,2004CB117403,2009CB118704,2010CB126300)、"863"计划(2001AA626030,2002AA626010,2006AA09Z445,2006AA100309,2006AA10A402)、国家自然科学基金(30170726,30671616,U0631008,30871938,31072239)、中国科学院院长基金(院基计字391号)、中国科学院特别基金(STZ-00-13)、淡水生态与生物技术国家重点实验室基金(1999FB12,2001FB08,085B161F01)、国家支撑计划(2006BAD03B05)和农业公益性行业科研专项基金(200803013)、湖北省自然科学基金(96J042)、湖北省创新群体基金(2004ABC005)等。

最后,要特别感谢父亲张汝瑞,父爱如山厚重如斯;感谢母亲洪菊芳,母爱如歌礼赞生命;感谢我们的两个女儿桂朗与桂暲,敏学乐群见贤思齐,使我们此生不虚!

张奇亚 桂建芳 **2011年12**月于武汉东湖之畔

Preface

In comparison with cells, viruses are very tiny, and must replicate within the host cells. Because of these unique properties, the specialized techniques and equipments are required to study them. Usually, they are only observed by microscope (e.g. electron microscope). Most of scholars have been pursuing an ideal realm that the virus structure, viral life activity, and their interaction with host cells are well witnessed in real-time. Researches on aquatic viruses in organisms and water environment not only allow us to better understand virus infection, morphogenesis and transmission mechanism, potential treatment of viral diseases, but also are beneficial for evaluating and exploiting bio-resources, pursuing virus origins and the relation with the hosts, and realizing the significant impact and biogeochemical cycles of aquatic viruses on freshwater and marine ecosystems.

Aquatic viruses include aquatic animal viruses, aquatic plant viruses and aquatic microorganism viruses, which are generally intracellular parasites in various aquatic organisms. In recent years, along with large number of viroplankton are found in marine and freshwater, aquatic viruses also include some free-floating viruses in water environments.

Aquatic virology is a subset of cross-disciplinary of hydrobiology and virology and a relatively young discipline. It originates from fish virology that has begun to form since 1950s. From 1950s to 1960s, fish virology was fast developed as cell culture and electron microscopy techniques were used for virus researches in fish, especially, as some fish cell lines were applied to fish virus isolation.

Along with rapid development of global aquaculture and frequent emerging of infectious diseases during the 1960s and 1970s, a variety of lethal virus diseases were identified in fish. In 1988, the first monograph of fish virology, "Fish Viruses



and Fish Viral Disease" written by American professor Wolf K., was published by Cornell University Press (New York). Twenty years later, we published the first textbook of "Aquatic Virology" (Beijing, Higher Education Press, 2008). During the past 50 years, a remarkable advance has occurred in the field of aquatic virology, but it is still lagging behind medical virology.

In this book, the authors attempt to trace the intellectual history of aquatic virology through direct microscope images (most of images had not yet been published) and application of the related techniques, such as electron microscope observation and biochemical analysis, by which simple images can succeed to recreate the most important insight, and perspicuous notation can represent meaningful propositions. Each image may be as a research case in aquatic virology, and will contribute to the relationship understanding between virus and the infected aquatic organism. Moreover, this book will offer a unique vision and lead its reader to a more profound and broader realm of aquatic virology.

Department of Virology at Wuhan University is the first department in the field of virology established in China. Since one (Qi-Ya Zhang) of the authors entered the Department, I soon felt that I would be indissolubly bound together with the discipline of virology, and have had a long cherished dream to compose and publish an atlas on aquatic virology. After graduation, I was luck assigned to Wuhan Institute of Virology, the Chinese Academy of Sciences. Like most of young scientists who had just entered the new field, however, I was unable to see any possibilities beyond the walls of our own temple. It was very regretted that just a few of knowledge was accumulated.

Under recommendation and help of my classmates at Wuhan University, Dr. Ming-Jie Jin, Dr. Xian-Qiang Li and Dr. Xiang-Dong Fu, I traveled long distance across the Pacific Ocean to USA for postgraduate education and scientific training at over 30 years old. Firstly, I got my master's degree from Medical College of Ohio (MCO) under guidance of Prof. Keith D. Garlid. Then, I joined the laboratory of Prof. Michael G. Rosenfeld (a fellow of American Academy of Sciences) in University of California, San Diego (UCSD) for further biotechnological training and research.

At the same time, the other author, Jian-Fang Gui, was also working with Prof. Xiang-Dong Fu of UCSD for the mechanism study of RNA processing and cell cycle regulation. Also, the beautiful beach and fascinating scenery in San Diego brought both blooms of our scholar and life concerns. In 1994, we cherished real vision for serving our country by the learned knowledge, and returned to the Institute of Hydrobiology, the Chinese Academy of Sciences. Here, the abundant academic environment has given us an excellent research opportunity for aquatic virology.

We would like to express our deepest gratitude to several famous scientists and members of the Chinese Academy of Sciences for supporting our initial research in aquatic virology. Prof. Yi-Yu Chen, the Institute director of the Chinese Academy of Sciences at that time, and then serving as Vice-President of the Chinese Academy of Sciences and President of National Natural Science Foundation of China (NSFC), offered the position and chance for fish virology research, and provided the initial research foundation. Prof. Zuo-Yan Zhu, the director of State Key Laboratory of Freshwater Ecology and Biotechnology (FEBL) at that time, and then serving as the Institute director of the Chinese Academy of Sciences and Vice-President of NSFC, provided the FEBL foundation for fish virology research. Prof. Jian-Kang Liu, the honorary director of the Institute of the Chinese Academy of Sciences, help us to review and correct the first aquatic virus manuscript we submitted to *Chinese Science Bulletin*. Prof. Wen-Xian Cao,



the President of Chinese Society of Ichthyology, gave us valuable suggestions for our research.

We would also like to express our gratitude to my Ph. D. advisors, Academician Yun-Fen Shen and Prof. Wei-Jun Wang. They devoted special attention to the education and encouraged us to learn more and work hard, which made us to confirm that the solid fruits in autumn come from the glorious flowers in spring. Moreover, we would also like to thank the present Institute director of the Chinese Academy of Sciences, Academician Jin-Dong Zhao, who allowed us to participate in the presided project (Foundation of Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-EW-Z-3). Thanks for his leadership and support, which has exploited a new opportunity for further expanding the topic of aquatic virology.

Many special thanks are owed to our team members and colleagues. They are Xiu-Ping Yuan, Zheng-Qiu Li, Rong Zhu, Zhong-Yuan Chen, Yu-Lin Jiang, Yan Li, Hong Liu, Xiao-Chun Luo, Feng Xiao, Jian Xie, Hui-Ying Sheng, Hong-Mei Ruan, Shou-Ying Zeng, Yan-Ming Liu, Chang-Sheng Du, Ai-Jun Lü, Wei Sun, Da-Li Miao, Wei Xu, Xiao-Hong Huang, Jian-Jun Tao, You-Hua Huang, Yusin Kim (post-Doc from North Korea), Zhe Zhao, Guang-Zhou Zhou, Fei Ke, E-Bin Gao, Lang Gui, Xiao-Ying Lei, Li-Bo He, Ruo-Lin Zhu, Shun Li, Chao Pei, San-Hua Li, Tong Ou, Xiao-Chan Gao, Jun Wang, Bi-Yu Xiang, and others. Over the years, whether we have been running through busy roads or working hard in the tranquil laboratory, which let's realize that improvement of aquatic virology knowledge is a continuous process of learning. We learn together so that more knowledge is able to enhance our mutual understanding. We should work even harder and will strive to make greater contributions to the progress of aquatic virology.

A leap of only one steed can't stride across ten steps, but ten nags through

perseverance are able to reach their destination (referred from the "Xunzi: Encouraging Learning"). The authors make the greatest attempts to collect multiple ideas and to gather the topic essences in the field of aquatic virology through accumulating knowledge from widely learning and continuously studying, and finally choose these images (or plates) for the atlas from more than ten thousands. Although it contains some shortcomings and needs for further improvement, the atlas records its unusual history of the new interdiscipline of aquatic virology, and concentrates a lot of contribution by numerous scientists and scholars. Here, we are looking forward to rapid advance in this discipline.

When the atlas is going to publication, we would also like to express our sincere thanks to Huan-Chun Chen, member of Chinese Academy of Engineering and Prof. of Huazhong Agricultural University, Wen-Xin Li, Prof. of Wuhan University, and to Ying-Hua Chen, Prof. of Tsinghua University, for their recommendations to the committee of National Fund for Academic Publication in Science Technology. Thanks to the editors at Science Press, Jing Wang and Jing Luo for their expert editing, and thanks to everyone who has provided academic and professional support during the atlas publication course.

Many thanks are owed to these foundations for their supports: National Fund for Academic Publication in Science and Technology (2010-c-002), National Program on Key Basic Research Project (973 Program, G1999053908, 2004CB117403, 2009CB118704, 2010CB126300), National High Technology Research and Development Program of China (863 Program, 2001AA626030, 2002AA626010, 2006AA09Z445, 2006AA100309, 2006AA10A402), National Natural Science Foundation of China (30170726, 30671616, U0631008, 30871938, 31072239), Knowledge Innovation Project of the Chinese Academy of Sciences (KSCX2-SW-302, KSCX2-YW-N-021, KSCX2-



YW-G-075-19, KSCX2-EW-Z-3), Presidential Foundation of the Chinese Academy of Sciences (1996-391), Special Foundation of the Chinese Academy of Sciences (STZ-00-13), Foundation of State Key Laboratory of Freshwater Ecology and Biotechnology (1999FB12, 2001FB08, 085B161F01), National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2006BAD03B05) and Special Fund for Agro-scientific Research in the Public Interest (200803013), Natural Science Foundation of Hubei Province of China (96J042) and Innovation group project of Hubei Province (2004ABC005).

Finally, we'd like to express our deepest thanks to father Ru-Rui Zhang for father's love being as high as mountain, and to mother Jü-Fang Hong for mother's love being like a poem. Thanks our daughters Lang Gui and Toni-Tong Gui, because they are good at learning from anyone and emulate virtuous and talented persons better than themselves, which make us to whisper that we have not lived in vain.

Qi-Ya Zhang & Jian-Fang Gui Dec. 2011 in Wuhan

目 录

前言

第一部分 水生病毒病症及水生病毒学技术

第一章	水生生物病毒病症状	2
	1.1 江豚疱疹样病毒病	3
	1.2 中华鳖病毒病	5
	1.3 沼泽绿牛蛙病毒病	8
	1.4 大鲵疑似病毒病	0
	1.5 中华鲟病毒病	1
	1.6 胭脂鱼病毒病	3
	1.7 长吻鮠病毒病	6
	1.8 斑点叉尾鮰病毒病	7
	1.9 鲤病毒病1	9
	1.10 牙鲆病毒病	20
	1.11 草鱼病毒病	22
^	1.12 鳜病毒病2	25
	1.13 加州鲈病毒病	26
	1.14 黄鳝病毒病	27
	1.15 大菱鲆病毒病	29
	1.16 黄颡鱼疑似病毒病	32



	1.18 近江牡蛎疑似病毒病
	1.19 浮丝藻病毒病
	1.20 鱼腥藻病毒病40
	1.21 铜绿微囊藻病毒病
第二章	水生动物的细胞与组织培养 47
	2.1 内脏组织原代培养
	2.2 鱼类皮肤组织原代培养
	2.3 细胞传代培养
	2.4 新建细胞系核型分析
	2.5 细胞碱性磷酸酶染色
	2.6 扫描电子显微镜观察69
第三章	鱼类病毒单克隆抗体的制备与应用71
	3.1 细胞融合72
	3.2 杂交瘤细胞的筛选
	3.3 免疫印迹分析
	3.4 单克隆抗体与流式细胞术79
	3.5 单克隆抗体免疫组化检测 80
	3.6 单克隆抗体免疫荧光观察83
第四章	水生病毒的检测 86
	4.1 细胞化学检测87
	4.2 组织化学检测100
	4.3 细胞集落形成测验105

	4.4 病毒侵染后细胞骨架结构的变化	108
	4.5 RNA干扰调节病毒基因的表达及其荧光检测 ······	114
	4.6 病毒基因产物与细胞器在宿主细胞中的共定位	116
	4.7 免疫扩散检验与酶联免疫检测	121
	4.8 流式细胞计量检测	123
	4.9 电泳图谱分析	124
	4.10 水生病毒蛋白质高级结构的预测	130
	4.11 水生病毒基因组组织结构	133
	4.12 水生病毒的基因功能解析	139
	4.13 含EGFP重组病毒的构建及其细胞病变空斑的识别 ·······	143
	4.14 噬藻体的检测	146
	第二部分 水生哺乳动物病毒	
第五章	江豚疱疹样病毒	156
2011	12100/1312 17 173 -3	
RANT	第三部分 爬行动物病毒	
	75—1175 IC 13 43 13 13 43	
第六章	中华鳖病毒	172
	6.1 超薄切片的透射电子显微镜观察	173
	6.2 负染电镜图	181



第四部分 两栖动物病毒

第七章	蛙虹彩病毒 184
	7.1 细胞病变185
	7.2 病毒加工厂 (病毒基质)形成192
	7.3 病毒装配
	7.4 空衣壳及异常病毒颗粒209
	7.5 病毒在宿主细胞中的分布220
	7.6 病毒释放240
	7.7 免疫电子显微镜观察246
	7.8 纯化的蛙虹彩病毒负染电镜图250
第八章	大鲵病毒样颗粒
	第五部分 鱼虾类动物病毒
第九章	中华鲟病毒样颗粒 256
第十章	胭脂鱼弹状病毒261
	10.1 细胞感染263
	10.2 包涵体270

牙鲆淋巴囊肿病毒与正粘样病毒颗粒 275 11.1 淋巴囊肿病毒中国分离株 276