文章编号: 1000-0615(2019)07-1647-14 DOI: 10.11964/jfc.20181011508

致急性肝胰腺坏死病副溶血弧菌(Vp_{AHPND})自然感染三疣梭子蟹

郝景伟[1,2], 高保全[1,2], 王 崇[1,2], 孟宪亮[1], 万晓媛[1], 李小平[1,2], 刘 萍[1,2], 张庆利[2*]

(1. 中国水产科学研究院黄海水产研究所, 青岛海洋科学与技术国家实验室, 海洋渔业科学与食物产出过程功能实验室, 农业农村部海水养殖病害防治重点实验室, 青岛市海水养殖流行病学与生物安保重点实验室, 山东 青岛 266071;
2. 上海海洋大学水产与生命学院, 上海 201306)

摘要: 近年来包括急性肝胰腺坏死病(AHPND)在内的多种新发疫病的流行, 使我国甲壳类养殖业遭受了严重的经济损失。为了筛查导致山东潍坊某养殖场中一虾蟹混养池塘内患病三疣梭子蟹感染的可能病原, 本研究采用分子生物学检测方法, 对三疣梭子蟹样品进行了白斑综合征病毒(WSSV)、传染性皮下及造血组织坏死病毒(IHHNV)、虾血细胞虹彩病毒(SHIV)、致急性肝胰腺坏死病副溶血孤菌(Vp_{AHPND})、虾肝肠胞虫(EHP)、偷死野田村病毒(CMNV)、黄头病毒(YHV)和肝胰腺细小病毒(HPV)等8种病原的检测, 并对样品进行了组织病理和原位杂交分析。分子生物学检测结果显示, 患病三疣梭子蟹样品呈Vp_{AHPND}阳性, 而呈现WSSV、IHHNV、SHIV、EHP、CMNV、YHV和HPV阴性。对样品进行Vp_{AHPND}套式PCR第二轮扩增产物的序列测定、比对和进化树分析, 结果显示, 扩增产物序列与致病副溶血弧菌质粒上$pirA^{vp}$毒力基因片段具有99%的同源性, 该序列与已报道的多个致病副溶血弧菌*PirA*聚在进化树的同一主分支上。组织病理学分析显示, 患病三疣梭子蟹的肝胰腺小管上皮细胞坏死, 心肌纤维呈溶解样病变, 鳃丝上皮柱突细胞明显坏死, 胸神经节的神经细胞损伤严重, 并且这些组织中还可见大量的细胞核固缩现象; 原位杂交结果显示, 肝胰腺、心肌、鳃组织及胸神经节中的病变部位均存在Vp_{AHPND}探针的蓝紫色杂交信号。以上表明, 虾蟹混养池塘中三疣梭子蟹在自然状态下感染了Vp_{AHPND}, 并导致肝胰腺、心肌、鳃和胸神经节发生了严重病理损伤。本研究首次在养殖三疣梭子蟹中检测到Vp_{AHPND}感染并揭示了感染所致的病理变化, 相关结果为揭示Vp_{AHPND}自然宿主种类和养殖三疣梭子蟹病害防控提供了基础信息。

关键词: 三疣梭子蟹; 急性肝胰腺坏死病(AHPND); 致急性肝胰腺坏死病副溶血弧菌(Vp_{AHPND}); 生长缓慢; 组织病理分析; 原位杂交

中图分类号: S 945.6 **文献标志码:** A

急性肝胰腺坏死病(acute hepatopancreatic necrosis disease, AHPND), 又被称为早期死亡综合征(early mortality syndrome, EMS)[1-2], 是近年来严重危害对虾养殖的一种新发疫病。该病2010年前后首先在越南和中国养殖的斑节对虾(*Peneus monodon*)和凡纳滨对虾(*Litopenaeus vannamei*)中

收稿日期: 2018-10-25 **修回日期:** 2019-01-09

资助项目: 国家重点研发计划(2017YFC1404503); 农业农村部农业国际合作交流项目——"一带一路"热带国家水产养殖科技创新合作; 中国水产科学研究院黄海水产研究所基本科研业务费专项(20603022019003)

通信作者: 张庆利, E-mail: zhangql@ysfri.ac.cn

被发现[3-5]，随后传播到东南亚的马来西亚、泰国、菲律宾以及中美洲的墨西哥等地[6-7]，使全球对虾养殖业每年损失近10亿美元[8-9]，严重影响了对虾产业的发展。对于该疫病的病原，至今还未有明确的定论，目前比较公认的病原是一种独特的副溶血性弧菌分离株(*Vibrio parahaemolyticus* causing AHPND，Vp_{AHPND})，该副溶血性弧菌携带含有二元Pir样毒素基因($pirA^{vp}$和$pirB^{vp}$)的69 kbp质粒(pAP1)[10-12]，该质粒可编码PirA和PirB毒素进而对养殖对虾具有致死性[13-15]。

最近的研究显示，越南南部患病对虾中分离的哈维氏弧菌(*V. harveyi*-KC13.17.5)可导致AHPND的类似症状[16]；分离自我国上海的一株欧文氏弧菌(*V. owensii* SH-14)也能引起凡纳滨对虾发生AHPND[17-19]；一株分离自拉丁美洲国家的坎贝氏弧菌(*V. campbellii* LA16-V1)也携带包含*pirAB* 基因的毒性质粒[20]，同样导致养殖凡纳滨对虾发生AHPND；从我国养殖对虾中分离得到的一株携带$pirA^{vp}$和$pirB^{vp}$基因的坎贝氏弧菌(VC_{AHPND}-20130629003S01)，同样具有导致对虾罹患AHPND的能力[21-22]。上述结果表明，携带有毒力基因$pirA^{vp}$和$pirB^{vp}$的多种弧菌，包括副溶血性弧菌、哈维氏弧菌、欧文氏弧菌和坎贝氏弧菌，感染对虾后均能使其发生AHPND [18]。

2016年，AHPND作为一种新发疫病被世界动物卫生组织(OIE)水生动物疫病目录收录。在该疫病病原的分子检测方面，除亚太水产养殖网络中心(Network of Aquaculture Centres in Asia-Pacific, NACA)公布的AP1、AP2和AP3引物外[23]，OIE公布了检测灵敏度更高、用于套式PCR检测的AP4引物[11, 24-25]。各国研究者基于上述PCR方法、病理学和病原基因组分析方法，对AHPND以及致AHPND的多种弧菌开展了广泛的研究，但目前关于AHPND的研究多集中在养殖对虾中，尚未见其他甲壳类动物感染AHPND病原的报道。Ding等[26]在患肝胰腺坏死病(HPND)的淡水养殖中华绒螯蟹(*Eriocheir sinensis*)中观测到微孢子虫，并推测微孢子虫感染可能与HPND有关；而杨宗英等[27]认为，中华绒螯蟹肝胰腺坏死病为非生物性疾病。到目前为止，尚未有证据表明淡水养殖中华绒螯蟹HPND与海水养殖动物AHPND间存在相关性。

三疣梭子蟹(*Portunus trituberculatus*)隶属于节肢动物门(Arthropoda)、甲壳纲(Crustacea)、十足目(Decapoda)、梭子蟹科(Portunidae)、梭子蟹属(*Portunus*)，广泛分布于中国、日本、朝鲜及马来西亚群岛等海域[28]。三疣梭子蟹肉嫩味美、营养价值高，备受人们的喜爱，已逐渐成为我国海水养殖的主要经济种类之一。山东潍坊一水产养殖企业虾蟹混养池塘中的三疣梭子蟹在发生AHPND后表现异常，并出现生长明显变慢的症状。本研究对上述三疣梭子蟹进行了已知甲壳类病原携带或感染情况检测，并在检出Vp_{AHPND}阳性的基础上对三疣梭子蟹组织样品进行了组织病理学和原位杂交分析，以期为揭示养殖三疣梭子蟹自然感染Vp_{AHPND}后所致组织病理变化，明确AHPND在养殖甲壳类中的传播流行规律提供基础信息。

1 材料与方法

1.1 样品采集与处理

患病三疣梭子蟹来自于山东潍坊市某养殖场，发病个体为Ⅶ期幼蟹，全甲宽约5~7 cm。取患病个体的肝胰腺、心脏、鳃丝和胸神经节等组织，分3份分别保存于95%乙醇、RNAstore样本保存液[天根生化科技(北京)有限公司]和4%多聚甲醛固定液(4%PFA)中，用于后续的核酸检测、组织病理学和分子病理学分析。4%PFA固定液中的样本固定24 h后，更换为70%的乙醇进行长期保存，留作组织病理切片及原位杂交实验使用。从发病池塘共采集了52份三疣梭子蟹样品用于分析。

1.2 组织病理切片制作

将保存在4%PFA固定液中的样本按照经典的组织学分析方法进行组织脱水、透明、浸蜡和包埋处理[29]，进而进行石蜡切片、展片及烘片，每份样品制备3张厚度为3 μm的石蜡切片：第1张切片进行苏木精和伊红(H.E)染色用于组织病理观察；第2张切片用于地高辛(DIG)标记的Vp_{AHPND} RNA探针进行组织原位杂交；第3张切片用于无探针的原位杂交阴性对照。将H.E染色的切片和原位杂交显色后的切片置于Nikon Eclipse E80i光学显微镜(Nikon, 日本)下观察并拍照。

1.3 组织核酸提取

将保存在95%乙醇溶液中的组织样本取出，先用无RNA酶水清洗，用吸水纸吸去多余水分后匀浆研磨，利用海洋动物组织基因组DNA提

取试剂盒[天根生化科技(北京)有限公司]提取样品的DNA；将保存在RNAstore样本保存液中的组织利用无RNA酶水清洗，并用吸水纸吸去多余水分，然后采用RNAiso plus[宝生物工程(大连)有限公司]法抽提样品总RNA。随后通过NanoDrop 2000 (Thermo Scientific，Waltham，MA，USA)测量所制备DNA和RNA的浓度和纯度。

1.4　多种病原的核酸检测分析

根据世界动物卫生组织《水生动物疾病诊断手册》(OIE，2017 版)中相应检测方法，对三疣梭子蟹样品中传染性皮下及造血组织坏死病毒(infectious hypodermal and hematopoietic necrosis virus, IHHNV)、黄头病毒(yellow head virus, YHV)、白斑综合征病毒(white spot syndrome virus, WSSV)、肝胰腺细小病毒(hepatopancreatic parvovirus, HPV)和致急性肝胰腺坏死病的副溶血弧菌(Vp_{AHPND})的存在情况进行PCR或套式PCR检测(表1)；根据文献报道的方法对样品中偷死野田村病毒(covert mortality nodavirus, CMNV)、虾肝肠胞虫(enterocytozoon hepatopenaei, EHP)和虾血细胞虹彩病毒(shrimp hemocyte iridescent virus, SHIV)的存在情况进行PCR或套式PCR检测[30-31](表1)。PCR产物在2%琼脂糖凝胶中电泳，利用凝胶成像仪(上海培清科技有限公司)拍照；扩增产物经切胶回收后送生工生物工程(上海)股份有限公司测序。

1.5　组织原位杂交探针制备

首先利用引物AP4-F1/R1(表2)通过PCR扩增Vp_{AHPND} *pirA*基因上一长度为1 269 bp的片段，然后用引物AHPND_Sen/Anti进一步扩增该片段内长度为247 bp的片段作为AHPND原位杂交探针的靶基因片段，参考此前文献报道的方法制备Vp_{AHPND}的RNA探针[32]：将AHPND原位杂交探针的靶基因片段插入pBluescript II SK+质粒，把重组质粒导入载体菌克隆后提取质粒并进行测序验证，对序列验证正确的重组质粒进行扩大培养并制备大量质粒，然后用限制性内切酶*Pst* I和*Hin*d Ⅲ将上述重组质粒线性化；以线性化的载体质粒为模板，采用T7 RNA聚合酶转录出DIG标记的Vp_{AHPND} RNA探针。

1.6　组织原位杂交

根据此前报道的原位杂交(ISH)方法[33-34]，利用上述制备的RNA探针对样品切片进行ISH分析，主要步骤：①用二甲苯和乙醇对切片进行脱蜡和水化，然后用4% PFA-PBS缓冲液对切片进行室温固定，随后依次利用PBS缓冲液、0.2 mol/L HCl进行处理和漂洗，进而利用10 μg/mL蛋白酶K消化；②每张切片加100~200 ng探针，在48~50 °C条件下进行杂交孵育；③利用1∶1 000混合的抗体/Blocking buffer对杂交后切片进行抗体孵育；④利用PBST缓冲液洗涤和AP缓冲液浸润后，每张切片加200 μL BCIP/NBT底物显色液进行避光显色，观察到合适强度的杂交信号后用PBST缓冲液冲洗终止显色；⑤对显色后的杂交切片进行核固红复染着色。利用显微镜(Nikon Eclipse E80i)观察原位杂交切片并拍照。上述过程中以不加探针的切片为空白对照，同步进行组织原位杂交。

2　结果

2.1　患病三疣梭子蟹症状

2017年8月，山东潍坊某养殖场的虾蟹混养池塘内中国明对虾(*Fenneropenaeus chinensis*)发生AHPND，随后池塘中混养的三疣梭子蟹也出现异常和发病，发病症状主要表现为生命活力减弱、行动迟缓、10%~20%的个体生长缓慢、脱壳困难、个体大小不均，大的即正常三疣梭子蟹全甲宽为9~11 cm，小的即患病三疣梭子蟹全甲宽仅为5~7 cm，大小个体的甲壳颜色无明显差异(图1)。

2.2　多种病原的检测

采用OIE推荐的WSSV、IHHNV、YHV、HPV和Vp_{AHPND}检测方法，以及根据文献报道的CMNV、EHP和SHIV检测方法对三疣梭子蟹样品进行分子生物学检测分析，发现这些样品中呈现WSSV、IHHNV、SHIV、EHP 、CMNV、YHV和HPV等7种病原PCR或套式PCR检测的阴性，而呈现Vp_{AHPND}套式PCR检测的阳性(图2，图3)。套式PCR的检测结果显示，发病池塘所采集的52份三疣梭子蟹样品中Vp_{AHPND}的阳性检出率为57%。

2.3　Vp_{AHPND}阳性片段测序及其系统发育分析

对三疣梭子蟹样品(编号为WF_20170906004)的Vp_{AHPND}检测第二轮PCR扩增片段进行基因序列测定，将所测得序列利用BLAST进行比对分析，结果显示，扩增片段序列与GenBank中公布的$pirA^{vp}$基因(GenBank登录号分别为KU145400.1、KU145399.1和KU145398.1)相应区段的相似度均为99%。

表 1 用于病原检测的PCR/套式PCR引物

Tab. 1 Primers sequences for PCR detection of pathogens

病原 pathogen	引物名称 primer name	引物序列 (5′-3′) primer sequences (5′-3′)	T_m值/°C T_m value	GC含量/% GC content	片段长度/bp length of amplicons
WSSV	146F1	ACTACTAACTTCAGCCTATCTAG	47.8	39.1	1 447
	146R1	TAATGCGGGTGTAATGTTCTTACGA	63.1	40.0	
	146F2	GTAACTGCCCCTTCCATCTCCA	62.9	54.5	941
	146R2	TACGGCAGCTGCTGCACCTTGT	67.7	59.1	
IHHNV	IHHNV-389F	CGGAACACAACCCGACTTTA	58.5	50.0	389
	IHHNV-389R	GGCCAAGACCAAAATACGAA	57.8	45.0	
	IHHNV-309F	TCCAACACTTAGTCAAAACCAA	55.3	36.4	309
	IHHNV-309R	TGTCTGCTACGATGATTATCCA	55.9	40.9	
SHIV	IV-F	GGGCGGGAGATGGTGTTAGAT	62.6	57.1	457
	IV-R	TCGTTTCGGTACGAAGATGTA	56.1	42.9	
	IV-NF	CGGGAAACGATTCGTATTGGG	64.1	52.4	129
	IV-NR	TTGCTTGATCGGCATCCTTGA	63.8	47.6	
Vp_{AHPND}	AP4-F1	ATGAGTAACAATATAAAACATGAAAC	46.3	23.1	1 269
	AP4-R1	ACGATTTCGACGTTCCCCAA	56.8	50.0	
	AP4-F2	TTGAGAATACGGGACGTGGG	57.1	55.0	230
	AP4-R2	GTTAGTCATGTGAGCACCTTC	53.2	47.6	
EHP	SWP_1F	TTGCAGAGTGTTGTTAAGGGTTT	58.7	39.1	514
	SWP_1R	CACGATGTGTCTTTGCAATTTTC	60.1	39.1	
	SWP_2F	TTGGCGGCACAATTCTCAAACA	66.0	45.5	148
	SWP_2R	GCTGTTTGTCTCCAACTGTATTTGA	60.6	40.0	
CMNV	CMNV-F1	AAATACGGCGATGACG	49.4	50.0	619
	CMNV-R1	ACGAAGTGCCCACAGAC	50.4	58.8	
	CMNV-F2	TCGCGTATTCGTGGAT	49.5	50.0	413
	CMNV-R2	TAGGGTCAAAAGGTGTAGT	46.5	42.1	
YHV	YHV-GY5	GAGCTGGAATTCAGTGAGAGAACA	60.4	45.8	794
	YHV-GY1	GACATCACTCCAGACAACATCTG	56.8	47.8	
	YHV-GY4	GTGAAGTCCATGTGTGTGAGACG	60.1	52.2	
	YHV-GY2	CATCTGTCCAGAAGGCGTCTATGA	63.3	50.0	YHV：277，GAV：406
	YHV-Y3	ACGCTCTGTGACAAGCATGAAGTT	63.0	45.8	
	YHV-G6	GTAGTAGAGACGAGTGACACCTAT	52.0	45.8	
HPV	HPV F1	GGTGATGTGGAGGAGAGA	51.8	52.6	628
	HPV R1	GTAACTATCGCCGCCAAC	53.5	55.5	

将所测得序列提交NCBI数据库进行BLASTX比对，发现该基因对应氨基酸序列与“PirA(*V. parahaemolyticus*)(GenBank登录号为AOS51074.1)”相应区段的相似度为97%，与“坎贝氏弧菌(GenBank登录号为WP_086370857.1)”序列相应区段的相似度为96%(表3)。将来自三疣梭子蟹样品基因序列(WF_20170906004)所对应的氨基酸序列、BLASTX比对结果涉及的部分典型菌株对应序列进行系统进化树分析，结果显示，WF_ 20170906004对应氨基酸序列与Vp_{AHPND}的PirA同属于一条主分支，且亲缘关系较近，与其他弧菌来源的氨基酸序列亲缘关系较远(图4)。上述多序列比对及系统进化树分析结果说明，编号为WF_20170906004的三疣梭子蟹样品中携带或感染了致AHPND的

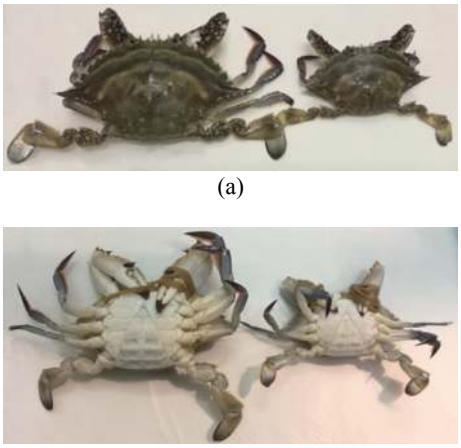
表 2　组织原位杂交探针合成中用到的引物

Tab. 2　Primers used for synthesis of the Vp_{AHPND} RNA probe

引物名称 primer name	引物序列 (5′-3′) primer sequences (5′-3′)	T_m值/°C T_m value	GC含量/% GC content	片段长度/bp length of amplicons
AP4-F1	ATGAGTAACAATATAAAACATGAAAC	46.3	23.1	1 269
AP4-R1	ACGATTTCGACGTTCCCCAA	56.8	50.0	
AHPND_Sen	CTACTGCAGTTGAGAATACGGGACGTGGG	64.6	55.2	247
AHPND_Anti	CAGAAGCTTGTTAGTCATGTGAGCACCTTC	61.1	46.7	

注：序列中灰色背景标出的“CTGCAG”为*Pst* I限制性酶切位点；灰色背景标出的“AAGCTT”为*Hin*d Ⅲ限制性酶切位点；下划线标出的序列“CTA”和“CAG”分别为保护性碱基

Notes: the sequence of “CTGCAG” with gray background indicated the recognition site of restriction enzyme of *Pst* I; the sequence of “AAGCTT” with gray background indicated the recognition site of restriction enzyme of *Hin*d Ⅲ, the underlined sequences of “CTA” and “CAG” were protective nucleotides for the recognition sites of restriction enzymes, respectively



(a)

(b)

图 1　正常与患病三疣梭子蟹外观对比

(a)背面观；(b)腹面观

Fig. 1　Difference of appearance of the normal and diseased individual of *P. trituberculatus*

(a) dorsal view; (b) ventral view

副溶血弧菌。

2.4　发病三疣梭子蟹的组织病理和原位杂交结果

从发病三疣梭子蟹肝胰腺组织的病理切片中可以观察到，部分肝胰腺小管结构损伤严重，管壁上皮细胞坏死、崩解，且出现严重的核固缩(图5-a，b)。肝胰腺组织的原位杂交结果显示，肝胰腺小管上皮细胞的细胞质中出现大量蓝紫色或深紫色Vp_{AHPND}探针杂交信号(图5-c，d)，空白对照组肝胰腺组织切片中无杂交信号(图5-e，f)。

发病三疣梭子蟹心脏组织的病理切片显示，心肌组织中出现严重的多病灶炎症反应，心肌纤维坏死、排列松散不规整，心肌内存在大量血细胞浸润(图6-a，b)。心肌组织的原位杂交图片显示，心肌组织内多病灶炎症反应和肌肉坏死部位有大量蓝紫色或深紫色的Vp_{AHPND}探针杂交信号(图6-c，d)，而空白对照组的心肌组织中无杂交信号(图6-e，f)。

患病三疣梭子蟹胸神经节组织的病理切片显示，胸神经节内神经细胞存在坏死现象，神经纤维排列松散，神经组织中血淋巴浸润明显，血细胞和神经细胞的细胞核固缩严重(图7-a，b)。从胸神经节组织的原位杂交图片可以看出，神经节内神经细胞的细胞质和浸润的血细胞内能够观察到蓝紫色或深紫色的Vp_{AHPND}探针杂交信号(图7-c，d)，而空白对照组的神经节组织中未出现杂交信号(图7-e，f)。

从发病三疣梭子蟹鳃组织的病理切片中可以观察到，次级鳃丝的上皮柱突细胞损伤严重，导致鳃丝内结构紊乱，另外鳃丝内有大量血细胞浸润，血细胞细胞核固缩明显(图8-a，b)。鳃组织的原位杂交切片显示，在鳃丝上皮柱突细胞和浸润的血细胞内能够清晰观察到蓝紫色或深紫色的Vp_{AHPND}探针杂交信号(图8-c，d)。对照组鳃丝中未观察到杂交信号(图8-e，f)。

3　讨论

近年来，随着养殖规模的扩大及养殖集约化程度的不断提高，三疣梭子蟹的养殖病害问题日渐突出，严重危害养殖业的健康发展，也引起从业者和研究人员的重视[35-38]。AHPND自2009年暴发以来，在我国沿海地区广泛传播和流行，使我国对虾养殖业遭受了严重的经济损失[6, 14, 18]。但目前为止，我国养殖三疣梭子蟹中尚未有检出AHPND病原的报道。

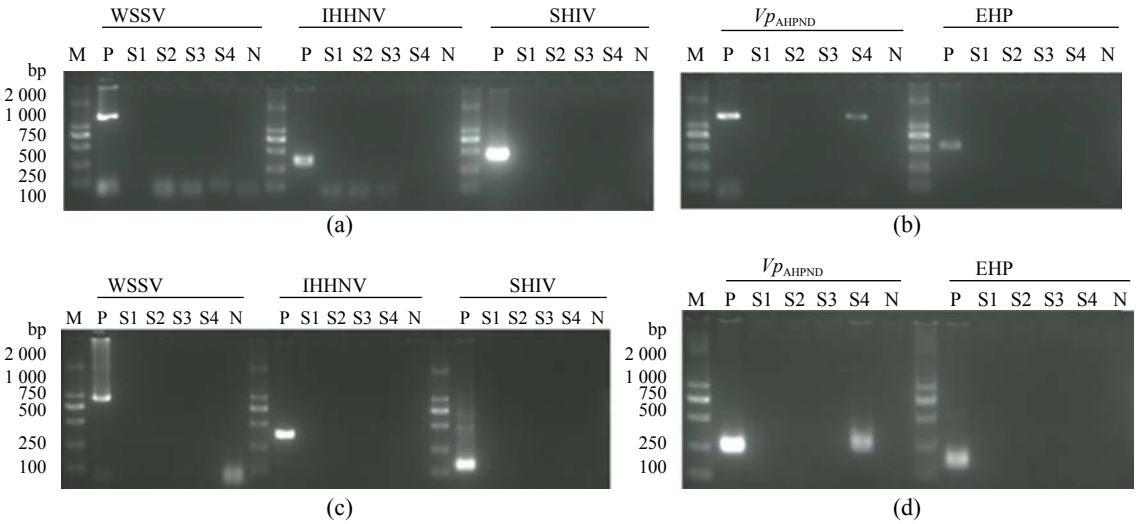


图 2 套式PCR检测三疣梭子蟹样品中WSSV、IHHNV、SHIV、Vp_{AHPND}和EHP电泳图

(a)、(b). 套式PCR第一轮PCR产物电泳图，(c)、(d). 套式PCR第二轮PCR产物电泳图；M. DL 2000 Marker；P. 阳性对照；N. 阴性对照；S1、S2、S3和S4. 山东潍坊三疣梭子蟹样品；下同

Fig. 2 Electropherogram of the nested-PCR for WSSV, IHHNV, SHIV, Vp_{AHPND} and EHP detection in samples of *P. trituberculatus*

(a), (b). electropherograms of the first round PCR product of nested-PCR; (c), (d). electropherograms of the second round PCR product of nested-PCR; M. DL 2000 Marker; P. positive control; N. negative control; S1, S2, S3 and S4. samples of *P. trituberculatus* from Weifang City, Shandong Province; the same below

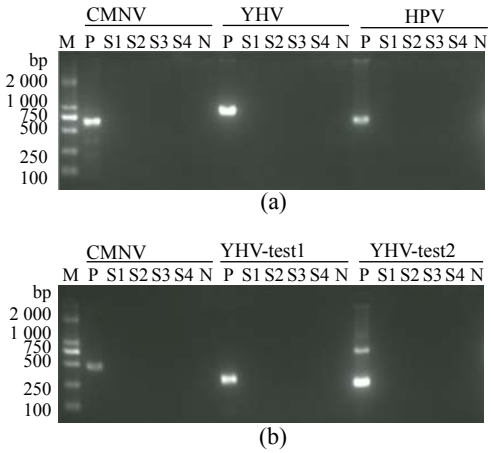


图 3 套式PCR检测三疣梭子蟹样品中CMNV、YHV和HPV电泳图

(a) 套式PCR第一轮PCR产物电泳图，(b) 套式PCR第二轮PCR产物电泳图

Fig. 3 Electropherogram of the nested-PCR for CMNV, YHV and HPV detection in samples of *P. trituberculatus*

(a) electropherograms of the first round PCR product of nested-PCR,
(b) electropherograms of the second round PCR product of nested-PCR

山东潍坊某虾蟹混养池塘养殖中国明对虾发生AHPND后，本研究对后续发病的三疣梭子蟹样品进行了分子生物学分析。检测结果显示，该池塘中发病三疣梭子蟹呈现IHHNV、YHV、HPV、WSSV、CMNV、EHP和SHIV等7种病原PCR或套式PCR检测的阴性，而呈现Vp_{AHPND}套式PCR检测的阳性；套式PCR结果显示，从该养殖场发病池塘采集的52份三疣梭子蟹样品中，Vp_{AHPND}的阳性检出率达57%；对Vp_{AHPND}套式PCR扩增产物进行核酸序列测定和多序列比对发现，来自三疣梭子蟹阳性片段的核酸序列与Vp_{AHPND}的$pirA^{vp}$基因具有99%的同源性；基于上述序列进行的系统进化树分析显示，来自三疣梭子蟹的$pirA^{vp}$基因的氨基酸序列与GenBank中已知可导致AHPND的多种弧菌的相应序列聚为一支。上述结果说明该三疣梭子蟹可能携带或感染了Vp_{AHPND}。

染病三疣梭子蟹的组织病理学分析表明，病蟹肝胰腺小管管壁细胞崩解，小管结构损伤严重，部分肝胰腺小管管壁变薄，肝胰腺小管上皮细胞坏死明显，出现严重的核固缩。发病三疣梭子蟹肝胰腺组织的这种病理变化，与此前报道的患AHPND凡纳滨对虾肝胰腺小管上皮细胞脱落、肝胰腺盲管上皮细胞坏死、细胞核固缩，后期肝胰腺盲管间或盲管内发生血细胞浸润[1-2, 5]等病理变化较为类似。Ding等[26]和杨宗英等[27]研究显示，中华绒螯蟹患HPND后其肝胰腺组织会出现不同程度的病理损伤，如肝胰腺组

表 3　三疣梭子蟹 Vp_{AHPND} *pirA*^{vp}基因编码的氨基酸序列与其他弧菌氨基酸序列的相似性

Tab. 3　Similarity of the amino acid sequence of Vp_{AHPND} *pirA*^{vp} from *P. trituberculatus* with other amino acid sequences of bacteria from GenBank

序列名称 amino acid sequences of *Vibrio* from GenBank	GenBank 登录号 GenBank no.	序列相似度/% similarity
PirA (*V. parahaemolyticus*)	AOS51074.1	97
hypothetical protein (*V. harveyi* group)	WP_023622799.1	97
hypothetical protein (*V. campbellii*)	WP_086370857.1	96
hypothetical protein (*V. campbellii*)	WP_045384433.1	64
hypothetical protein (*Shewanella violacea*)	WP_013050436.1	39
hypothetical protein (*Pectobacterium carotovorum*)	WP_039278756.1	29
c-type cytochrome biogenesis protein CcmI (*Pantoea stewartii*)	WP_006120094.1	36
hypothetical protein (*Yersinia enterocolitica*)	WP_083159813.1	30
hypothetical protein (*Y. aldovae*)	WP_053093377.1	30
hypothetical protein (*Y. intermedia*)	WP_050086265.1	30
hypothetical protein (*Y. intermedia*)	WP_032905692.1	30
hypothetical protein (*Yersinia*)	WP_042569345.1	30

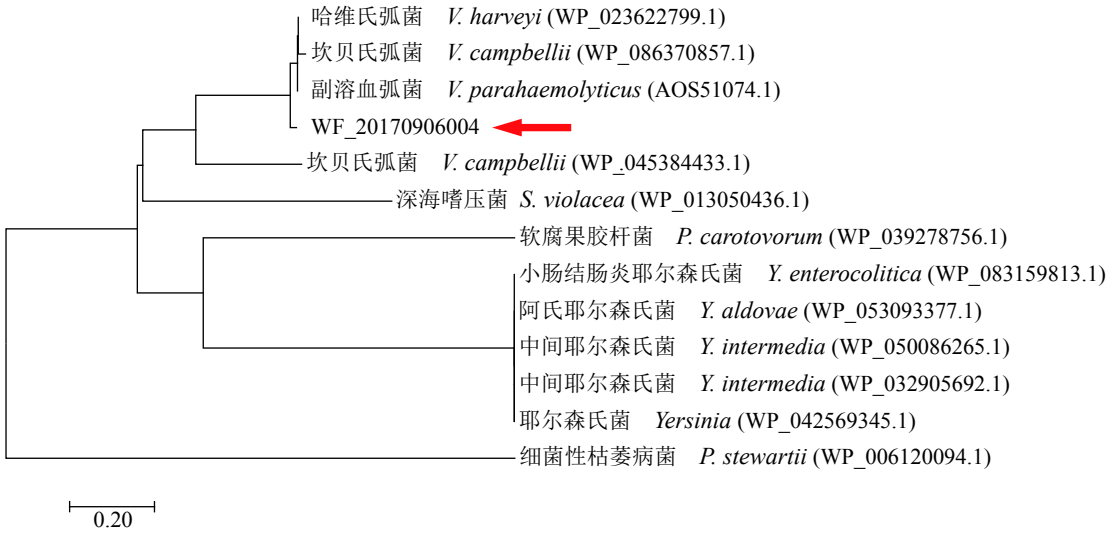


图 4　基于三疣梭子蟹 Vp_{AHPND} *pirA*序列及GenBank中其同源序列所构建的系统发育树

进化树采用MEGA 5.0程序通过邻接法生成

Fig. 4　Phylogenetic tree constructed by using deduced amino acid sequences of *pirA* from Vp_{AHPND} positive samples of *P. trituberculatus* and other bacteria species from GenBank

The tree was generated by the neighbor-joining method using the MEGA 5.0 program

织中单层上皮细胞空泡化、肝细胞排列紊乱、转运泡和空泡的数量增多、体积增大等，随着病情的加重，甚至出现肝胰腺小管基膜破裂、内容物外流和细胞核解体的情况。患AHPND三疣梭子蟹和患HPND中华绒螯蟹的肝胰腺上皮细胞均出现明显的坏死，但前人报道的患HPND中华绒螯蟹肝胰腺上皮细胞病变中未见有细胞核固缩的现象，这与AHPND所致三疣梭子蟹肝胰腺上皮细胞细胞核出现严重核固缩现象存在差异。另外，Ding等[26]研究认为微孢子虫感染可能与HPND有关，而杨宗英等[27]研究认为中华绒螯蟹HPND为非生物性疾病，这些结果似乎表明淡

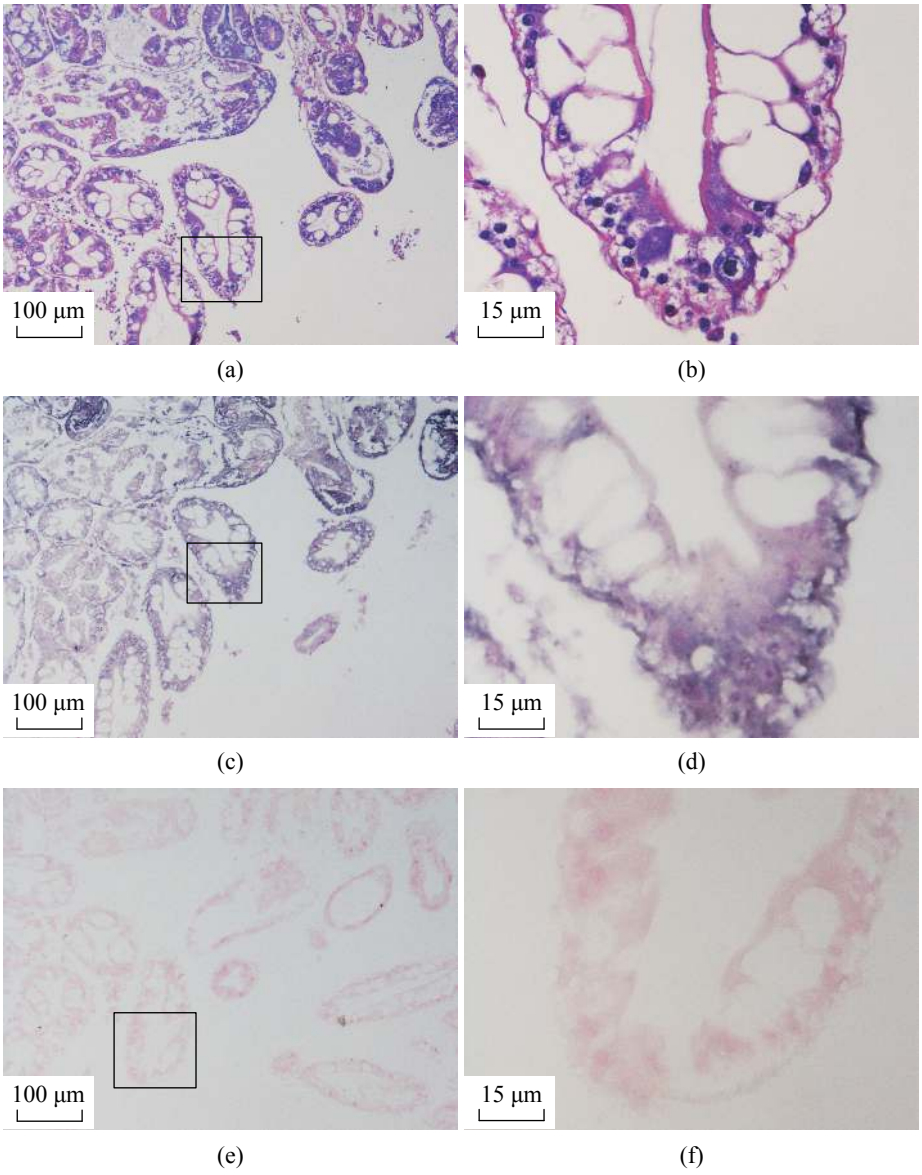


图 5 发病三疣梭子蟹肝胰腺的组织病理和原位杂交图片

(a)发病三疣梭子蟹肝胰腺组织的H.E染色病理图片；(b)为(a)中黑框内区域的放大图片；(c)发病三疣梭子蟹肝胰腺组织原位杂交图片；(d)为(c)中黑框内区域的放大图片；(e)为对照组的组织原位杂交图片；(f)为(e)中黑框内区域的放大图片

Fig. 5 Micrographs of H.E staining and *in situ* hybridization for hepatopancreas of *P. trituberculatus* naturally infected by Vp_{AHPND}

(a) micrographs of histopathological H.E staining for section of the hepatopancreas of *P. trituberculatus*; (b) magnified micrograph of the area in the black frame in (a); (c) micrographs of ISH for section of the hepatopancreas of *P. trituberculatus*; (d) magnified micrograph of the area in the black frame in (c); (e) micrographs of ISH for section of the hepatopancreas of the *P. trituberculatus* without the Vp_{AHPND} probe; (f) magnified micrograph of the area in the black frame in (e)

水养殖中华绒螯蟹HPND与海水养殖动物AHPND并不是一类病害。

本研究还分析了染病三疣梭子蟹心肌、胸神经节和鳃丝组织的病理变化，心肌内炎症反应严重，心肌纤维坏死、排列松散不规整，胸神经节内神经细胞坏死、神经纤维排列松散，次级鳃丝的上皮柱突细胞损伤严重、鳃丝内结构紊乱。同时这些组织中均存在大量血细胞浸润和细胞核固缩的现象，这与其他携带毒力基因$pirA^{vp}$和$pirB^{vp}$的弧菌感染对虾导致靶组织血细胞浸润和炎症反应严重的现象也较为相似[19, 21-22]。

本实验针对染病三疣梭子蟹不同组织的病理学分析，揭示出Vp_{AHPND}感染可导致病蟹肝胰腺、心肌、胸神经节和鳃组织发生明显的病理变化，

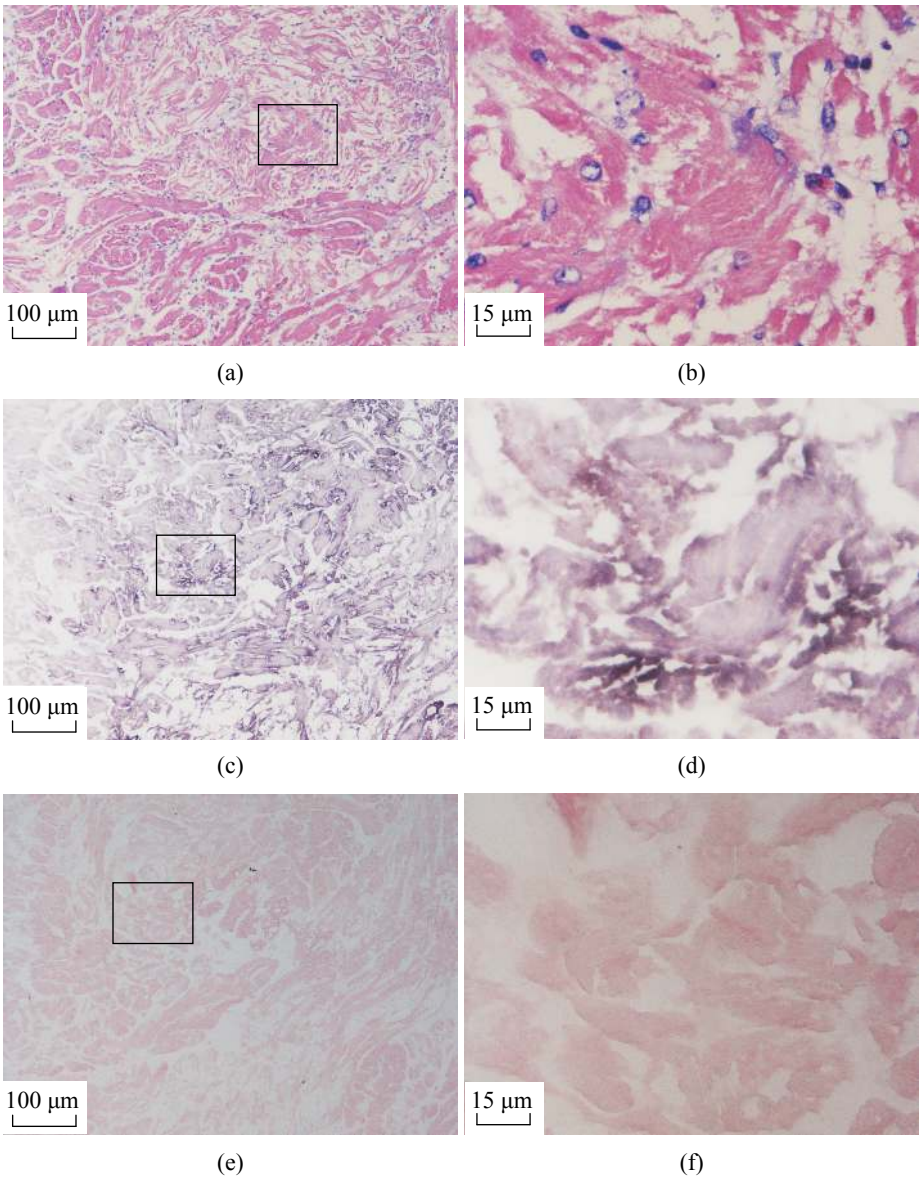


图 6　发病三疣梭子蟹心脏组织的病理和原位杂交图片

(a)发病三疣梭子蟹心脏组织的H.E染色病理图片；(b)为(a)中黑框内区域的放大图片；(c)发病三疣梭子蟹心脏组织的原位杂交图片；(d)为(c)中黑框内区域的放大图片；(e)为对照组的组织原位杂交图片；(f)为(e)中黑框内区域的放大图片

Fig. 6　Micrographs of H.E staining and *in situ* hybridization for section of heart of *P. trituberculatus* naturally infected by Vp_{AHPND}

(a) micrographs of histopathological H.E staining for section of the heart of *P. trituberculatus*; (b) magnified micrograph of the area inside the black frame in (a); (c) micrographs of ISH for section of the heart of *P. trituberculatus*; (d) magnified micrograph of the area within the black frame in (c); (e) micrographs of ISH for section of the heart of *P. trituberculatus* without the Vp_{AHPND} probe; (f) magnified micrograph of the area within the black frame in (e)

并且原位杂交的结果显示，这些病变组织中均存在很强的Vp_{AHPND}探针杂交信号。该结果进一步证实，虾蟹混养池塘中养殖中国明对虾发生AHPND后，染病三疣梭子蟹体内所发生的组织损伤是由Vp_{AHPND}感染所引起。此前的研究表明，生物胺作为神经递质/神经调质或激素对甲壳动物的蜕皮、生殖活动等具有重要的调节作用，这类物质广布于甲壳动物的中枢神经系统和外周器官内[39-40]；本实验发现Vp_{AHPND}感染可导致三疣梭子蟹胸神经节损伤和较为严重的病理变化，初步推测这种组织损伤可能影响了病蟹体内生物胺的分泌，进而影响了其蜕壳活动和正常生长。

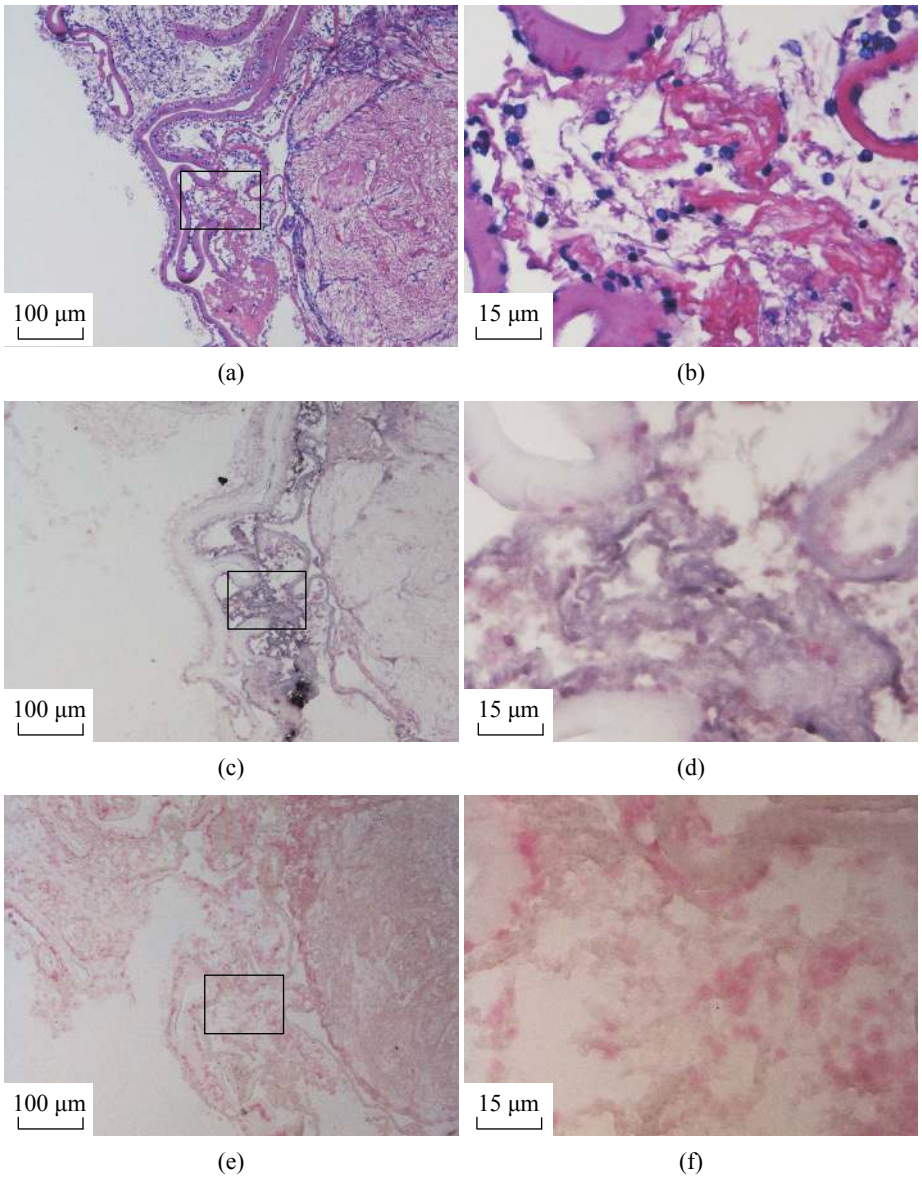


图 7 发病三疣梭子蟹胸神经节组织的病理和原位杂交图片

(a)发病三疣梭子蟹胸神经节组织的H.E染色病理图片；(b)为(a)中黑框内区域的放大图片；(c)发病三疣梭子蟹胸神经节组织的原位杂交图片；(d)为(c)中黑框内区域的放大图片；(e)为对照组的组织原位杂交图片；(f)为(e)中黑框内区域的放大图片

Fig. 7 Micrographs of H.E staining and *in situ* hybridization for thoracic central ganglion of *P. trituberculatus* naturally infected by Vp_{AHPND}

(a) micrographs of histopathological H.E staining for section of the thoracic central ganglion of *P. trituberculatus*; (b) magnified micrograph of the area inside the black frame in (a); (c) micrographs of ISH for section of the thoracic central ganglion of *P. trituberculatus*; (d) magnified micrograph of the area within the black frame in (c); (e) micrographs of ISH for section of the thoracic central ganglion of *P. trituberculatus* without the Vp_{AHPND} probe; (f) magnified micrograph of the area within the black frame in (e)

同时，本研究针对染病三疣梭子蟹不同组织所开展的原位杂交结果同样证实，虾蟹混养池塘中三疣梭子蟹在自然条件下感染了Vp_{AHPND}，并引起多个组织的严重病理损伤。

本研究证实，虾蟹混养池塘中的三疣梭子蟹在中国明对虾发生AHPND后，自然感染上了AHPND的病原Vp_{AHPND}，导致三疣梭子蟹多组织产生严重的病理损伤，推测病蟹中枢神经节损伤可能是引起染病三疣梭子蟹生长缓慢的原因。本研究在养殖的三疣梭子蟹中鉴定到Vp_{AHPND}感染，为揭示Vp_{AHPND}自然宿主种类和加强养殖三疣梭子蟹病害防控提供了参考。

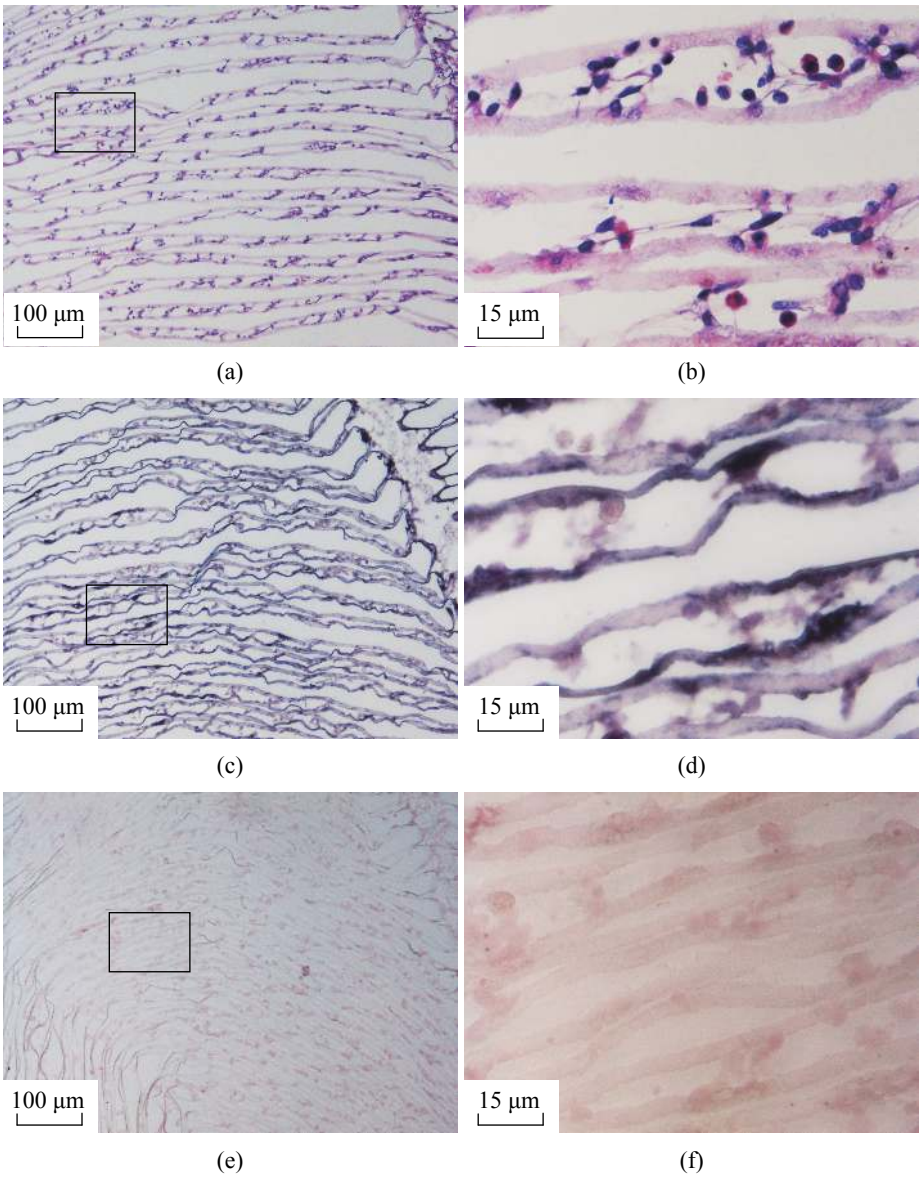


图 8　发病三疣梭子蟹鳃组织的病理和原位杂交图片

(a)发病三疣梭子蟹鳃组织的H.E染色病理图片；(b)为(a)中黑框内区域的放大图片；(c)发病三疣梭子蟹鳃组织的原位杂交图片；(d)为(c)中黑框内区域的放大图片；(e)为对照组的组织原位杂交图片；(f)为(e)中黑框内区域的放大图片

Fig. 8　Micrographs of H.E staining and *in situ* hybridization for gill of *P. trituberculatus* naturally infected by Vp_{AHPND}

(a) micrographs of histopathological H.E staining for section of the gill of *P. trituberculatus*; (b) magnified micrograph of the area inside the black frame in (a); (c) micrographs of ISH for section of the gill of *P. trituberculatus*; (d) magnified micrograph of the area inside the black frame in (c); (e) micrographs of ISH for section of the gill of *P. trituberculatus* without the Vp_{AHPND} probe; (f) magnified micrograph of the area inside the black frame in (e)

参考文献：

[1] NACA. Asia pacific emergency regional consultation on the emerging shrimp disease: early mortality syndrome (EMS)/acute hepatopancreatic necrosis syndrome (AHPNS)[R]. Bangkok, Thailand: Network of Aquaculture Centres in Asia-Pacific, 2012.

[2] Lightner D V, Redman R M, Pantoja C R, *et al*. Early mortality syndrome affects shrimp in Asia[J]. Global Aquaculture Advocate, 2012, 15(1): 40.

[3] NACA-FAO. Quarterly aquatic animal disease report (Asia and Pacific region)[R]. Bangkok: Network of Aquaculture Centres in Asia-Pacific, 2011.

[4] Flegel T W. Historic emergence, impact and current status of shrimp pathogens in Asia[J]. Journal of Inverte-

brate Pathology, 2012, 110(2): 166-173.

[5] 孙明玉,冯博,张昭寰,等. 引起凡纳滨对虾急性肝胰腺坏死病的副溶血弧菌MLST新序列型[J]. 水产学报, 2018, 42(3): 410-418.
Sun M Y,Feng B,Zhang Z H,*et al*. New sequence type isolates of AHPND-causing *Vibrio parahaemolyticus* from *Litopenaeus vannamei* by multilocus sequence typing[J]. Journal of Fisheries of China, 2018, 42(3): 410-418(in Chinese).

[6] Huang J. Experience in EMS/AHPNS from China[C]// NACA. The Asia Pacific emergency regional consultation on the emerging shrimp disease: early mortality Syndrome (EMS)/acute hepatopancreatic necrosis syndrome (AHPNS). Bangkok, Thailand: Network of Aquaculture Centres in Asia-Pacific, 2012.

[7] Thitamadee S, Prachumwat A, Srisala J, *et al*. Review of current disease threats for cultivated penaeid shrimp in Asia[J]. Aquaculture, 2016, 452: 69-87.

[8] Global Aquaculture Alliance. Cause of EMS shrimp disease identified: GAA news release[EB/OL]. (2013-05)[2018-09-09]. https://www.aquaculturealliance.org/blog/cause-of-ems-shrimp-disease-identified/.

[9] 孙明玉, 刘海泉, 冯博, 等. 对虾急性肝胰腺坏死症研究进展[C]//中国食品科学技术学会第十三届年会论文摘要集. 北京: 中国食品科学技术学会, 2016: 69-70.
Sun M Y, Liu H Q, Feng B, *et al*. Progress in research on acute hepatopancreatic necrosis disease (AHPND) of shrimp[C]/China Food Science and Technology Society Annual Conference, 2016: 69-70(in Chinese).

[10] Tran L, Nunan L, Redman R M, *et al*. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp[J]. Diseases of Aquatic Organisms, 2013, 105(1): 45-55.

[11] Yang Y T, Chen I T, Lee C T, *et al*. Draft genome sequences of four strains of *Vibrio parahaemolyticus*, three of which cause early mortality syndrome/acute hepatopancreatic necrosis disease in shrimp in China and Thailand[J]. Genome Announcements, 2014, 2(5): e00816-14.

[12] Sirikharin R, Taengchaiyaphum S, Sanguanrut P, *et al*. Characterization and PCR Detection of binary, pir-like toxins from *Vibrio parahaemolyticus* isolates that cause acute hepatopancreatic necrosis disease (AHPND) in shrimp[J]. PLoS One, 2015, 10(5): e0126987.

[13] Lee C T, Chen I T, Yang Y T, *et al*. The opportunistic marine pathogen *Vibrio parahaemolyticus* becomes virulent by acquiring a plasmid that expresses a deadly toxin[J]. Proceedings of the National Academy of Sciences of the United States of America, 2015, 112(34): 10798-10803.

[14] 唐小千, 徐洪森, 战文斌. 对虾急性肝胰腺坏死综合症研究进展[J]. 海洋湖沼通报, 2016(2): 90-93.
Tang X Q, Xu H S, Zhan W B. Advances in study of shrimp acute hepatopancreas necrosis syndrome[J]. Transactions of Oceanology and Limnology, 2016(2): 90-93(in Chinese).

[15] Theethakaew C, Nakamura S, Motooka D, *et al*. Plasmid dynamics in *Vibrio parahaemolyticus* strains related to shrimp acute hepatopancreatic necrosis syndrome (AHPNS)[J]. Infection, Genetics and Evolution, 2017, 51: 211-218.

[16] Kondo H, Van P T, Dang L T, *et al*. Draft genome sequence of non-*Vibrio parahaemolyticus* acute hepatopancreatic necrosis disease strain KC13.17.5, isolated from diseased shrimp in Vietnam[J]. Genome Announcement, 2015, 3(5): e00978-15.

[17] Liu L Y, Xiao J Z, Xia X M, *et al*. Draft genome sequence of *Vibrio owensii* Strain SH-14, which causes shrimp acute hepatopancreatic necrosis disease[J]. Genome Announcement, 2015, 3(6): e01395-15.

[18] Xiao J Z, Liu L Y, Ke Y Y, *et al*. Shrimp AHPND-causing plasmids encoding the PirAB toxins as mediated by *pir*AB-Tn903 are prevalent in various *Vibrio* species[J]. Scientific Reports, 2017, 7: 42177.

[19] Liu L Y, Xiao J Z, Zhang M M, *et al*. A *Vibrio owensii* strain as the causative agent of AHPND in cultured shrimp, *Litopenaeus vannamei*[J]. Journal of Invertebrate Pathology, 2018, 153: 156-164.

[20] Ahn Y S, Piamsomboon P, Tang K F J, *et al*. Complete genome sequence of acute hepatopancreatic necrosis disease-causing *Vibrio campbellii* LA16-V1, isolated from *Penaeus vannamei* cultured in a Latin American country[J]. Genome Announcements, 2017, 5(37).

[21] Dong X, Bi D X, Wang H L, *et al*. $pirAB^{VP}$-bearing *Vibrio parahaemolyticus* and *Vibrio campbellii* pathogens isolated from the same AHPND-affected pond possess highly similar pathogenic plasmids[J]. Frontiers in Microbiology, 2017, 8: 1859.

[22] Dong X, Wang H L, Zou P Z, *et al*. Complete genome sequence of *Vibrio campbellii* strain 20130629003S01 isolated from shrimp with acute hepatopancreatic necrosis disease[J]. Gut Pathogens, 2017, 9: 31.

[23] Flegel T W, Lo C F. Interim primers for specific det-

ection of bacterial isolates that cause acute hepatopancreatic necrosis disease (AHPND)[EB/OL]. (2014). https://www.was.org/Meetings/ShowAbstract.aspx?Id=32078.

[24] Dangtip S, Sirikharin R, Sanguanrut P, *et al*. AP4 method for two-tube nested PCR detection of AHPND isolates of *Vibrio parahaemolyticus*[J]. Aquaculture Reports, 2015, 2: 158-162.

[25] Arunrut N, Kampeera J, Sirithammajak S, *et al*. Sensitive visual detection of AHPND bacteria using loop-mediated isothermal amplification combined with DNA-functionalized gold nanoparticles as probes[J]. PLoS One, 2016, 11(3): e0151769.

[26] Ding Z, Meng Q, Liu H, *et al*. First case of hepatopancreatic necrosis disease in pond-reared Chinese mitten crab, *Eriocheir sinensis*, associated with microsporidian[J]. Journal of Fish Diseases, 2016, 39(9): 1043-1051.

[27] 杨宗英, 张一柳, 胡鲲, 等. 中华绒螯蟹肝胰腺坏死综合症病原及病理学研究[J]. 水生生物学报, 2018, 42(1): 17-25.
Yang Z Y, Zhang Y L, Hu K, *et al*. Etiological and histopathological study on hepatopancreatic necrosis syndrome in eriocheir sinensis[J]. Acta Hydrobiologica Sinica, 2018, 42(1): 17-25(in Chinese).

[28] 丁立云,付辉云,侯迎梅,等. 饲料磷脂对已交配和未交配雌性三疣梭子蟹卵巢发育、组织学和卵黄蛋白原表达的影响[J]. 水产学报, 2018, 42(3): 366-375.
Ding L Y, Fu H Y, Hou Y M, *et al*. Effects of dietary soy lecithin on the ovary development, histology and vitellogenin gene expression in mated and unmated female swimming crab (*Portunus trituberculatus*)[J]. Journal of Fisheries of China, 2018, 42(3): 366-375(in Chinese).

[29] Zhang Q L, Liu Q, Liu S, *et al*. A new nodavirus is associated with covert mortality disease of shrimp[J]. Journal of General Virology, 2014, 95(12): 2700-2709.

[30] Jaroenlak P, Sanguanrut P, Williams B A P, *et al*. A nested PCR assay to avoid false positive detection of the microsporidian *Enterocytozoon hepatopenaei* (EHP) in environmental samples in shrimp farms[J]. PLoS One, 2016, 11(11): e0166320.

[31] Zhang Q L, Xu T T, Wan X Y, *et al*. Prevalence and distribution of covert mortality nodavirus (CMNV) in cultured crustacean[J]. Virus Research, 2017, 233: 113-119.

[32] Nuovo G J, Plaia T W, Belinsky S A, *et al*. *In situ* detection of the hypermethylation-induced inactivation of the *p*16 gene as an early event in oncogenesis[J]. Proceedings of the National Academy of Sciences of the United States of America, 1999, 96(22): 12754-12759.

[33] Piette D, Hendrickx M, Willems E, *et al*. An optimized procedure for whole-mount *in situ* hybridization on mouse embryos and embryoid bodies[J]. Nature Protocols, 2008, 3(7): 1194-1201.

[34] Hamasaki K, Fukunaga K, Kitada S. Batch fecundity of the swimming crab *Portunus trituberculatus* (Brachyura: Portunidae)[J]. Aquaculture, 2006, 253(1-4): 359-365.

[35] 阎斌伦, 梁利国, 张晓君. 三疣梭子蟹主要病害研究进展[J]. 水产科技情报, 2010, 37(1): 29-33, 37.
Yan B L, Liang L G, Zhang X J. Prevention and cure of fish disease proceedings on main diseases of swimming crab *Portunus trituberculatus*[J]. Fisheries Science & Technology Information, 2010, 37(1): 29-33, 37(in Chinese).

[36] 王金凤. 寄生性甲藻血卵涡鞭虫流行病学及致病机理初探[D]. 青岛: 中国科学院大学(海洋研究所), 2017.
Wang J F. The epidemiology and pathogensis of the parasitic dinoflagellate *Hematodinium* sp. infecting marine crustaceans along the coat of China [D]. Qingdao, University of Chinese Academy of Sciences (Institute of Oceanography), 2017(in Chinese).

[37] 王厚红. 池塘虾蟹混养管理中存在的问题与措施[J]. 农业灾害研究, 2018(4): 97-98.
Wang H H. Problems and management measures of crayfish and crab polyculture in pond[J]. Journal of Agricultural Catastrophology, 2018(4): 97-98(in Chinese).

[38] 王元. 三脊尾白虾与三疣梭子蟹微孢子虫病的病原和病理[D]. 上海: 上海海洋大学, 2013.
Wang Y. The pathogen and pathology of microsporidiasis in ridgetail white prawn, *Exopalaemon carinicaud* and swimming crab, *Portunus trituberculatus*[D], Shanghai: Shanghai Ocean University, 2013 (in Chinese).

[39] Fingerman M, Nagabhushanam R, Sarojini R, *et al*. Biogenic amines in crustaceans: identification, localization, and roles[J]. Journal of Crustacean Biology, 1994, 14(3): 413-437.

[40] 叶海辉, 李少菁, 李祺福, 等. 生物胺对锯缘青蟹大颚器发育的调节作用[J]. 海洋科学, 2003, 27(4): 35-37.
Ye H J, Li S J, Li Q F, *et al*. Roles of biogenic amine in stimulating the development of the mandibular gland in mud crab, *Scylla serrata*[J]. Marine Science, 2003, 27(4): 35-37(in Chinese).

Natural infection of *Portunus trituberculatus* with acute hepatopancreas necrosis disease causing by *Vibrio parahaemolyticus* (Vp_{AHPND})

HAO Jingwei [1,2], GAO Baoquan [1,2], WANG Chong [1,2], MENG Xianliang [1], WAN Xiaoyuan [1], LI Xiaoping [1,2], LIU Ping [1,2], ZHANG Qingli [2*]

(1. *Function Laboratory for Marine Fisheries Science and Food Production Processes*, *Qingdao National Laboratory for Marine Science and Technology*, *Key Laboratory of Maricultural Organism Disease Control*, *Ministry of Agriculture and Rural Affairs*, *Qingdao Key Laboratory of Mariculture Epidemiology and Biosecurity*, *Yellow Sea Fisheries Research Institute*, *Chinese Academy of Fishery Sciences*, *Qingdao* 266071, *China*;
2. *College of Fisheries and Life Science*, *Shanghai Ocean University*, *Shanghai* 201306, *China*)

Abstract: The prevalence of multiple emerging diseases including acute hepatopancreatic necrosis disease (AHPND) has caused serious economic losses of crustacean aquaculture industry in China in last several years. In order to analyzing the possible pathogenic agents related to the slow growth of *Portunus trituberculatus* from Weifang City, Shandong Province, the presence and infection of nine suspected pathogens in the disease *P. trituberculatus* individuals were investigated by using the molecular detection methods in present study. The nine pathogens included white spot syndrome virus (WSSV), Infectious Hypodermal and hematopoietic necrosis virus (IHHNV), shrimp hemocyte iridescent virus (SHIV), AHPND-causing *Vibrio parahaemolyticus* (Vp_{AHPND}), enterocytozoon hepatopenaei (EHP), covert mortality nodavirus (CMNV), yellow head virus (YHV) and hepatopancreatic parvovirus (HPV). Meanwhile, histopathological analysis and *in situ* hybridization (ISH) were performed on the clinical samples. Results of molecular analysis showed that the *P. trituberculatus* samples were determined to be positive of Vp_{AHPND} and negative of other seven pathogens. Multiple sequence alignment based on the amplicons of Vp_{AHPND} nested-PCR of the samples revealed that sequences of the amplicons from *P. trituberculatus* samples shared 99% similarity to the $pirA^{vp}$ virulence gene from the pathogenic *V. parahaemolyticus*. Phylogenetic analysis indicated that the sequence from *P. trituberculatus* samples was clustered into the same branch with the reported PirA of pathogenic *V. parahaemolyticus*. Histological examination revealed necrosis of epithelial cells in hepatopancreas tubules, myonecrosis of cardiac muscle in heart, necrosis of epithelial columnar cells of gills, severe damage of nerve cell in the thoracic ganglion, as well as nuclear pyknosis in these tissues. Micrographs of ISH showed that blue-violet hybridization signals of the Vp_{AHPND} probes were present in the lesions of the hepatopancreas, myocardium, gills, and thoracic ganglia. The results indicated that the *P. trituberculatus* samples from the shrimp and crab polyculture ponds were naturally infected by Vp_{AHPND}, which caused serious pathological damages of the hepatopancreas, myocardium, gills, and thoracic ganglia. The present study revealed, for the first time, that Vp_{AHPND} infection in *P. trituberculatus* and the pathological changes caused by the infection. The results provided basic information for revealing the natural host species of Vp_{AHPND}, and for the prevention and control of AHPND of *P. trituberculatus*.

Key words: *Portunus trituberculatus*; acute hepatopancreatic necrosis disease (AHPND); AHPND-causing *Vibrio parahaemolyticus* (Vp_{AHPND}); slow-growing; histopathological analysis; *in situ* hybridization (ISH)

Corresponding author: ZHANG Qingli. E-mail: zhangql@ysfri.ac.cn

Funding projects: National R & D Program of China (2017YFC1404503); Projects of International Exchange and Cooperation in Agriculture, Ministry of Agriculture and Rural Affairs of China-Science, Technology and Innovation Cooperation in Aquaculture with Tropical Countries along the Belt and Road; Central Public-interest Scientific Institution Basal Research Fund, YSFRI, CAFS (20603022019003)