First Report of *Oryctes rhinoceros nudiovirus* (Coleoptera: Scarabaeidae) Causing Severe Disease in *Allomyrina dichotoma* in Korea

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**ABSTRACT.** *Oryctes rhinoceros nudiovirus* (*OrNV*) has been known to cause severe disease in coconut palm rhinoceros beetle, *Oryctes rhinoceros*, in Southeastern Asia and is used as a biological control to reduce the pest population. Here, we report for the first time that the *OrNV* may have landed on Korea and may be the major pathogen for diseased larvae of Korean horn beetle, *Allomyrina dichotoma*. After peroral inoculation, over 60% of infected larvae perished in 6 wk. This viral disease spreads very fast in several locations throughout Korea. This threat not only makes economic loss of local farms rearing *A. dichotoma* larvae but also may disturb the ecosystem by transmitting to wild *A. dichotoma*.

**Key Words:** Coleoptera, Scarabaeidae, diagnostics, virology, insect rearing

In global agriculture, the value of insect resources has been increased immensely, and the insect industry is now considered as a big market. Besides the traditional apiculture and sericulture industry, other insects are used for many purposes including pollinating activity, pet or educational purpose, animal feedstuffs, development of pharmaceuticals or cosmetics, natural enemy of harmful insects, and environmental cleanup. In Korea, the market size of this insect industry was estimated at 160 million dollars in 2012, and it is expected to increase up to 40 billion dollars by 2020. Among them, *Allomyrina dichotoma* is one of the strongest candidates for insect industry as medicinal purpose. *A. dichotoma* belongs to the order Coleoptera, family Scarabaeidae, and genus *Allomyrina*. The adult beetles range from 40 to 85 mm and suck out the sap of oak tree, whereas the larvae have three instar stages and feeds on rotting oak tree sawdust. Historically, Korean horn beetle has been used for traditional oriental medicine for various liver diseases and diabetes mellitus in Korea, and there are many reports that *A. dichotoma* larvae also have antineoplastic, antibacterial, and antioxidative effects (Taketa et al. 1986, Jeune et al. 2001, Sagisaka et al. 2001, Yamada et al. 2004, Choi et al. 2006, Kim et al. 2007, Lee and Lee 2009, Suh et al. 2010). Recently, it was reported that extracts from *A. dichotoma* larvae have also antiobesity and anti-Alzheimer activity (Chung et al. 2014, Kim et al. 2014).

*Oryctes rhinoceros nudiovirus* (*OrNV*) is a double-stranded DNA virus having an enveloped rod-shaped virion, which is about 200–235 nm in length and 100–120 nm in width (Huger 2005, Wang et al. 2011). *OrNV* causes severe disease in coconut palm rhinoceros beetle, *Oryctes rhinoceros*, in Southeastern Asia (Huger 2005, Ramle et al. 2005). The coconut palm rhinoceros beetle is a serious pest of coconut oil palm industry, and *OrNV* has been used as a biological control agent for the beetle. It was known that *OrNV* can also infect in other members of *Oryctes* genus, including *Oryctes monocrates* in Africa (Bedford 2013). The virus multiplies in the midgut, and fat body of infected larvae and the virus are released from the dead larvae on the breeding site.

In 2012, an incident was reported that *A. dichotoma* larvae being farmed died en masse in Cheongwon County, Korea. The appearances of diseased larvae were not like the symptom caused by infection of bacterial or fungal pathogens and the cause of death was suspected by viral disease. However, the viral pathogen was not identified. Since then, the disease has been reported from time to time, but in 2014, suddenly, several cases of the similar symptoms were reported nationwide. Here, we report for the first time that a virus, which seems to be *OrNV*, was identified in diseased larvae of *A. dichotoma*, and this viral disease spreads fast in several locations throughout Korea. Because unlike the coconut palm rhinoceros beetle, Korean horn beetle is not a pest but a good candidate for insect industry in Korea, this viral disease may become a serious threat for Korean farmers and insect industry.

**Materials and Methods**

**Virus Collection and DNA Isolation.** The diseased larvae were collected from several places throughout Korea including Cheongwon County, Youngdong County, Pocheon City, Yuseong District, and Gyeongsan City in 2014. The hemolymph was extracted through wound on a leg of a diseased larva, and the virus was purified with PEG virus precipitation kit (BioVision, Milpitas, CA). First, the hemolymph was centrifuged at 2,000 × *g* for 15 min at 4°C to remove cell debris, and the supernatant was passed through a cellulose nitrate membrane with pore size of 0.45 μm. Next, 2.5 ml of PEG solution A was added to 10 ml of the supernatant and refrigerated overnight. The virus–PEG mixture was centrifuged at 10,000 × *g* for 30 min at 4°C, and the viral pellet was dissolved in 20–100 μl of virus resuspension solution. For DNA isolation, hemolymph and midgut of diseased larvae were homogenized and centrifuged 2,000 × *g* for 15 min at 4°C to remove cell debris. Viral DNA was extracted with Wizard plus SV miniprep kit (Promega, Madison, WI) as instructed by the manufacturer.

**Oligodeoxyribonucleotide Design for Virus Diagnosis.** For diagnosis of the diseased *A. dichotoma* larvae, three pairs of primers were designed based on the *OrNV* genome (GenBank accession no. NC_011588). Primer AdV-F1 is 5′-TCCGGAATTACAGGACGCCAC-3′ corresponding from 58,961 to 58,981 bp of *OrNV* genome. Primer AdV-R1 is 5′-ATGCCGTACGAGAGTATAGGTCG-3′, corresponding from 59,604 to 59,582 bp. Amplification using primer pair AdV-F1 and -R1 yields 644 bp fragment of lef-8 gene (*OrNV* gp064). Primer AdV-F2 is 5′-TGGTGTGACGAGATAAGCTGTC-3′, corresponding from 23,249 to 23,273 bp, whereas primer AdV-R2 is 5′-TGGTGTGACGAGATAAGCTGTC-3′, corresponding from 23,853 to 23,832 bp. Amplification between primer AdV-F2 and -R2 produces the 605 bp fragment of GrBNV_gp76-like protein (*OrNV* gp025). Primer AdV-F3 is 5′-GGGTGTGACGAGATAAGCTGTC-3′ and corresponds from 48,009 to 48,030 bp. Primer AdV-R3
The viral DNA fragment amplified by AdV-F1/R1, -F2/R2, and -F3/R3 was isolated from agarose gel, and DNA sequences were determined with an Applied Biosystems 3730xl DNA Analyzer and this also accelerates the fast spread of the disease. Also, there is often observed that a healthy larva ingests a diseased cadaver along with the sawdust. Many farmers trade their larvae for crossbreeding, and the cadavers were diagnosed as OrNV infection by PCR with primers AdV-F1/R1, -F2/R2, and -F3/R3. Since it was first reported in 2012, the causation of mass loss of A. dichotoma larvae in Korea has been suspected as viral disease, and finally, it was turned out that that OrNV or at least OrNV-like virus may be the major pathogen for the viral disease. The PCR-based diagnose method makes it possible to detect the virus even in early stage of disease before symptom appears. Because in the farms rearing A. dichotoma larvae, dozens or hundreds of larvae grow together in a big plastic container filled with moist sawdust, a few viral-diseased larvae can easily infect the other larvae. Moreover, a cannibal behavior is often observed that a healthy larva ingests a diseased cadaver along with the sawdust. Many farmers trade their larvae for crossbreeding, and this also accelerates the fast spread of the disease. Also, there is growing concerns that this viral disease may be transmitted to the wild A. dichotoma because in some larvae farm located near mountain, the farmers lure the wild A. dichotoma for crossbreeding. Therefore, early

### Table 1. Three pairs of primers, AdV-F1, -R1, -F2, -R2, -F3, and -R3, were designed based on the OrNV genome for diagnosis of the diseased A. dichotoma larvae

| Primers  | GenBank accession no. | Primer sequence (5'→3') | Location         | Product (bp) |
|----------|-----------------------|--------------------------|------------------|--------------|
| AdV-F1   | KM233708              | TCCGGAAATTACAGGAGGACCAC  | lef-8 (YP_002321375) | 644          |
| AdV-R1   | KM233708              | ATGCGCTAGAGAGTTAGGTGCG   | lef-8 (YP_002321375) | 605          |
| AdV-F2   | KM233709              | TGGTAGCCCTATAGGACTGCTC   | GrBNV gp76-like protein (YP_002321336) | 644          |
| AdV-R2   | KM233709              | GGGTGGGACGAGGAAAACAGCC   | GrBNV gp76-like protein (YP_002321336) | 605          |
| AdV-F3   | KM233710              | GCAGCCGCTGTAATAATGGCGG   | Ribonucleotide reductase (YP_002321362) | 644          |
| AdV-R3   | KM233710              | GGCGCTGTAATAATGGCGG      | Ribonucleotide reductase (YP_002321362) | 605          |

The sequence data were submitted to GenBank (accession no. KM255708, KM255709, KM255710).

### Discussion

Since it was first reported in 2012, the causation of mass loss of A. dichotoma larvae in Korea has been suspected as viral disease, and finally, it was turned out that that OrNV or at least OrNV-like virus may be the major pathogen for the viral disease. The PCR-based diagnose method makes it possible to detect the virus even in early stage of disease before symptom appears. Because in the farms rearing A. dichotoma larvae, dozens or hundreds of larvae grow together in a big plastic container filled with moist sawdust, a few viral-diseased larvae can easily infect the other larvae. Moreover, a cannibal behavior is often observed that a healthy larva ingests a diseased cadaver along with the sawdust. Many farmers trade their larvae for crossbreeding, and this also accelerates the fast spread of the disease. Also, there is growing concerns that this viral disease may be transmitted to the wild A. dichotoma because in some larvae farm located near mountain, the farmers lure the wild A. dichotoma for crossbreeding. Therefore, early
Fig. 3. The amplification of OrNV with AdV primers is species specific. Lanes 1, 2, and 3 are amplification of virus-infected larvae with AdV-F1/R1, -F2/R2, and -F3/R3, respectively, while a bacterial pathogen, B. thuringiensis, causing disease in A. dichotoma, was not amplified by AdV primers (lanes 4–6). Positive control in lane 7 for B. thuringiensis amplification with primer Bt-1 and Bt-2 yielded the expected size of DNA band. Another bacterial pathogen, S. marcescens, was also not amplified by AdV primers (lanes 8–10), while for the positive control in lane 11, S. marcescens was amplified with luxF and -R, correctly. Furthermore, two fungal pathogens, M. anisopliae and B. bassiana, were not amplified by AdV primers (lanes 12–14 and lanes 16–18). The fungal pathogens were amplified by their own primers Nc-F/R and Bb-P1/P3 as positive controls (lanes 15 and 19).

detection and removal of the diseased larvae from the breeding cage are extremely important at this stage.

The identification of this virus is not clear yet, and full-genome sequencing of the virus is planned for comparison with OrNV genome. If it is turned out the origin of this virus is OrNV, a further study should be carried out immediately how this viral epidemic has landed on Korea and how to block the epidemic route.

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References Cited

Bedford, G. O. 2013. Biology and management of Palm Dynastid Beetles: recent advances. Annu. Rev. Entomol. 58: 353–372.

Choi, J. Y., Y. H. Je, and K. Y. Kim. 2004. GenBank direct submission. Accession numbers: AF237118.1.

Choi, Y. H., K. Lee, K. M. Yang, Y. M. Jeong, and J. S. Seo. 2006. Effect of larva extract of Allomyrina dichotoma on carbon tetrachloride-induced hepatotoxicity in mice. J. Korean Soc. Food Sci. Nutr. 35: 1349–1355.

Chung, M. Y., Y. I. Yoon, J. S. Hwang, T. W. Goo, and E. Y. Yun. 2014. Anti-obesity effect of Allomyrina dichotoma (Arthropoda: Insecta) larvae ethanol extract on 3T3-L1 adipocyte differentiation. Entomol. Res. 44: 9–16.

Huger, A. M. 2005. The Oryctes virus: its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, Oryctes rhinoceros (Coleoptera: Scarabaeidae). J. Invertebr. Pathol. 89: 78–84.

Jeune, K. H., M. Y. Jung, S. J. Cho, J. W. Lee, W. H. Park, S. H. Cho, S. H. Lee, and S. R. Chung. 2001. Immunomodulating effect of the lectin from Allomyrina dichotoma. Korean J. Pharmacognosy 32: 31–38.

Kim, D., J. Huh, G. C. You, S. C. Chae, O. S. Lee, H. B. Lee, J. B. Lee, and J. S. Kim. 2007. Allomyrina dichotoma larva extracts protect streptozotocin-induced oxidative cytotoxicity. J. Environ. Toxicol. 22: 349–355.

Kim, M., K. Youn, E. Y. Yun, J. S. Hwang, M. R. Ahn, W. S. Jeong, and M. Jun. 2014. Effects of solvent fractions of Allomyrina dichotoma larvae through the inhibition of in vitro BACE1 and β-amyloid(25–35)-induced toxicity in rat pheochromocytoma PC12 cells. Entomol. Res. 44: 23–30.

Lee, K., and J. Lee. 2009. Protective effect of Allomyrina dichotoma larva extract on tert-butyl hydroperoxide-induced oxidative hepatotoxicity. Korean J. Environ. Biol. 27: 230–236.

Ramle, M., M. B. Wahid, K. Norman, T. R. Glare, and T. A. Jackson. 2005. The incidence and use of Oryctes virus for control of rhinoceros beetle in oil palm plantations in Malaysia. J. Invertebr. Pathol. 89: 85–90.

Sagisaka, A., A. Miyanoshiba, J. Ishibashi, and M. Yamakawa. 2001. Purification, characterization and gene expression of glycine and proline-rich antibacterial protein family from larvae of beetle, Allomyrina dichotoma. Insect Mol. Biol. 10: 293–302.

Shin, T. Y., J. B. Choi, S. M. Bae, H. N. Koo, J. Y. Roh, Y. H. Je, B. R. Jin, and S. D. Woo. 2011. Characterization of Beauveria bassiana MoW1 isolated from pine sawyers, Monochamus saltuarius. J. Basic Microbiol. 51: 531–539.

Suh, H., S. Kim, K. Lee, S. Park, and S. Kang. 2010. Antioxidant activity of various solvent extracts from Allomyrina dichotoma (Arthropoda: Insecta) larvae. J. Photochem. Photobiol. B Biol. 99: 67–73.

Taketa, K., E. Ichikawa, K. Uemitsu, and T. Suzuki. 1986. Allomyrina dichotoma lectin-nonreactive α-fetoprotein in hepatocellular carcinoma and other tumors: comparison with Ricinus communis agglutinin-1. Cancer Lett. 31: 325–331.

Wang, Y., O. Bininda-Emonds, M. M. Oers, J. M. Vlak, and J. A. Jehle. 2011. The genome of Oryctes rhinoceros nudivirus provides novel insight into the evolution of nuclear arthropod-specific large circular double-stranded DNA viruses. Virus Genes 42: 444–456.

Yamada, M., K. Nakamura, H. Saido-Sakanaka, A. Asaoda, M. Yamakawa, T. Sameshima, M. Motobu, and Y. Hirota. 2004. Effect of modified oligopeptides from the beetle Allomyrina dichotoma on Escherichia coli infection in mice. J. Vet. Med. Sci. 66: 137–142.

Yamada, S., E. Ohashi, N. Agata, and K. Venkateswaran. 1999. Cloning and nucleotide sequence analysis of gyrB of Bacillus cereus, B. thuringiensis, B. mycoides, and B. anthracis and their application to the detection of B. cereus in rice. Appl. Environ. Microbiol. 65: 1483–1490.

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