

## Notes on the Indian wax scale, *Ceroplastes ceriferus* (Fabricius), from Korea (Hemiptera: Coccidae)

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### 한국산 뿔밀각지벌레에 대한 정리 (노린재목, 밀각지벌레과)

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**ABSTRACT:** The Indian wax scale, *Ceroplastes ceriferus* (Fabricius) is redescribed so that it can be distinguished from *Ceroplastes pseudoceriferus* Green, which it most nearly resembles and has been confused with in Korea. A dichotomous key, photographs, and DNA barcode information are also presented for identifying three *Ceroplastes* species from Korea.

**Key words:** *Ceroplastes ceriferus*, redescription, key, Korea

**조 록:** 뿔밀각지벌레(*Ceroplastes ceriferus* (Fabricius))는 형태적으로 유사한 *C. pseudoceriferus* Green과 혼동되어 동정되었으며, 이에 뿔밀각지벌레에 대한 형태적 특징을 재기재하였다. 이와 더불어 한국산 밀각지벌레屬의 3종을 동정하기 위한 검색표, 사진 및 DNA바코드 정보도 함께 제공하였다.

**검색어:** 뿔밀각지벌레, 재기재, 검색표, 한국

The genus *Ceroplastes* Gray are soft scale insects which have a characteristic thick wax test that covers the body of the adult female. These are most abundant in tropical and subtropical areas, but are known from all zoogeographic regions. Currently the genus contains approximately 139 species which have been reported worldwide (Ben-Dov *et al.*, 2012). In Korea, three species, *C. japonicus* Green, *C. pseudoceriferus* Green, and *C. rubens* Maskell, have been previously documented (Paik, 1978; Paik, 2000; Lee, 2010).

The first record of the Indian wax scale in Korea was confirmed in 1930 by Machida and Aoyama. This species was considered to be a pest of persimmon, apple, pear, and cherry trees by Kanda (1941). In addition, it was listed as a serious pest

of *Citrus* plants in Jeju, Korea (Paik, 1958), however, the name of the author of the species was incorrectly cited as Anderson in his paper. In the check list of insects from Korea, *C. ceriferus* was recorded under its common name, the Indian wax scale (Kwon *et al.*, 1994).

However, it has been cited as *Ceroplastes pseudoceriferus* Green in recently published literatures (Paik, 1978, 1986; Paik, 2000; Han *et al.*, 2002; Kwon and Han, 2003; Kwon *et al.*, 2005; Lee, 2010). In the illustrated flora and fauna of Korea published by Paik (1978), the illustration of what was labeled as *Ceroplastes pseudoceriferus* Green, probably represents a misidentification of *C. ceriferus*. Recently we carefully reexamined some specimens collected during the 1970's that had been identified as *C. pseudoceriferus* based upon his book through comparing them to specimens of *C. ceriferus* deposited at the Florida State Collection of Arthropods; however we determined

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that all of them were actually *C. ceriferus*. Therefore, we consider the species Paik (2000) documented as *C. pseudoceriferus* to be a misidentification of *C. ceriferus* (Fabricius). In addition, *C. ceriferus* was misidentified as *C. pseudoceriferus* by Kwon and Han (2003) and Kwon *et al.* (2005), and the mention of *C. pseudoceriferus* in the checklist of Korean insects (Lee, 2010), probably represents a misidentification of *C. ceriferus*.

In Korea, the Indian wax scale, *C. ceriferus* has been confused with *C. pseudoceriferus*. The purpose of this paper is 1) to redescribe *C. ceriferus* so that it can be compared with *C. pseudoceriferus* which is not known to occur in Korea, 2) to provide an identification key to Korean *Ceroplastes* species and photographs of their major characters, and 3) to show a genetic evidence to support the existence of two species, *C. ceriferus* and *C. pseudoceriferus*.

## Materials and Methods

### Morphological methods

Terminology for morphological structures used in this paper is that of Gimpel *et al.* (1974). Photographs were taken using AxioCam MRc camera through ZEISS Axio Imager M2 Microscope and a LEICA M165C microscope with Delta pix camera. All specimens for accurate identification were mounted on slide and are deposited in the Collection of Yeongnam Regional Office, Animal, Plant and Fisheries Quarantine and Inspection Agency (QIA) in Busan, Korea. An asterisk(\*) is used to indicate a new host record. Abbreviations are given as follows; GG: Gyeonggido; GB: Gyeongsangbukdo; GN: Gyeongsangnamdo; JB: Jeollabukdo; JN: Jeollanamdo; JJ: Jeju-do.

### Molecular methods

The collection information of *Ceroplastes* species are as follows (Table 1). With respect to the Korean three *Ceroplastes* species, the primers (Fwd\_seq: CCTTCAACTAATCA TAAA-AATATYAG, Rev\_seq: TAAACTTCTGGATGTCCAAAAAATCA) that were used to amplify a 658bp fragment of the mitochondrial cytochrome *c* oxidase subunit I gene (COI), for scale insects were designed by Park *et al.* (2010). All DNA was extracted from either dried or ethanol-fixed samples using a standard Glass Fibre extraction protocol (Ivanova *et al.*, 2006). PCR thermocycling was done under the following conditions: 2 min at 95C; 5 cycles of 40 sec at 94C, 40 sec at 45C, 70 sec at 72C; 40 cycles of 40 sec at 94C, 40 sec at 51C, 70 sec at 72C; 5 min at 72C; held at 4C. DNA sequences of specimens of *C. pseudoceriferus* from China were provided by Wu and all analyses were conducted at Beijing Forestry University. Sequences were aligned in the ClustalW2 (ver. 2.1). Also, Neighbour-joining analysis, implemented in MEGA (ver. 2.1), was employed to show a relationship of three species of *Ceroplastes* from Korea, including *C. pseudoceriferus* from China.

## Results and Discussion

### Description

***Ceroplastes ceriferus* (Fabricius), 1798** 뽕밀깍지벌레  
*Coccus ceriferus* Fabricius, 1798: 546.

**Field Characters.** Body covered by thick white (young female) to pinkish white (old female) wet wax, convex, circular or irregular in outline, with an anteriorly projecting horn. Plates

**Table 1.** Collection details of *Ceroplastes* species used in the molecular analyses

Species	Locality, number of samples, date, collector, and host
<i>Ceroplastes ceriferus</i>	Daedong-myeon, Gimhae (GN), 4 specimens, 27-ix-2005, S.J. Suh, on <i>Camellia japonica</i>
	Jungang-dong, Busan (GN), 3 specimens, 6-i-2012, S.J. Suh, on <i>Camellia japonica</i>
	Jungang-dong, Busan (GN), 3 specimens, 6-i-2012, S.J. Suh, on <i>Rhododendron indicum</i>
<i>Ceroplastes japonicus</i>	Jinhae (GN), 4 specimens, 8-v-2008, S.J. Suh, on <i>Diospyros kaki</i>
	Jungang-dong, Busan (GN), 3 specimens, 29-xii-2011, S.J. Suh, on <i>Camellia japonica</i>
<i>Ceroplastes pseudoceriferus</i>	Yunnan, Kunming, China, 1 specimen, 17-ix-2011, Ying-jie Zhang, on <i>Buxus megistophylla</i>
<i>Ceroplastes rubens</i>	Yeon-dong, Jeju (JJ), 2 specimens, 16-v-2006, S.J. Suh, on <i>Citrus</i> sp.
	Jungang-dong, Busan (GN), 4 specimens, 10-i-2012, S.J. Suh, on <i>Camellia japonica</i>

visible only on older females, without nuclei. Wax bands near both spiracles, anterior bands directed dorsally. Dry wax with filaments as follows: cephalic filament trifurcate; anterolateral and mediolateral simple; posterolateral bifurcate; caudal simple (Hamon and Williams, 1984).

**Slide-mounted Characters.** Dorsum: Without a mediadorsal clear area and with cephalic and posterolateral clear areas divided; dorsal setae predominantly cylindrical with rounded apices; with 1 ventral and 4 dorsal seta on each anal plate; with about 54 stigmatic setae laterad of each stigmatic furrow which are bullet-shaped to lanceolate, with pointed apices; with 1 dorsal submarginal band of filamentous ducts. Venter: Membranous with many cruciform pores; 1 ventral submarginal band of filamentous ducts; multilocular pores present on all abdominal segments and normally present near each coxa; with appendages unusually short, legs about 261  $\mu\text{m}$  long and antennae about 180  $\mu\text{m}$  long, legs without tibiotarsal sclerites, with unequal claw digitules; antennae 6-segmented (Gimpel *et al.*, 1974; Kawai, 1980).

**Material examined.** Korea. GG: Opo-ri, Gwangju, 4 specimens, on *Zizyphus jujuba* (Rhamnaceae), 16-vii-1998 (I.T. Park); Incheon, 17 specimens, on *Acer palmatum* (Aceraceae), 1-viii-1999; Seonhak-dong, Incheon, 2 specimens, on *Rhododendron schlippenbachii* (Ericaceae), 8-iii-2001 (H.J. Lee); Suwon, 5 specimens, on *Camellia japonica* (Theaceae), 27-ix-2005 (S.J. Suh); Anyang, 5 specimens, on *Euonymus japonicus* (Celastraceae), 1-i-2006 (S.J. Suh); Mampo-dong, Suwon, 3 specimens, on *Rhaphis excelsa* (Arecaceae), 29-ix-2006 (S.J. Suh); same data, except for on *Zizyphus jujuba* (Rhamnaceae). GB: Sangyeok-dong, Daegu, 10 specimens, on *Acer palmatum* (Aceraceae), 18-iv-2009 (S.J. Suh). GN: Jungang-dong, Busan, 16 specimens, on *Rhododendron indicum* (Ericaceae), 10-x-2001 (Y.H. Lee); Daedong-myeon, Gimhae, 4 specimens, on *Camellia japonica* (Theaceae), 5-ix-2005 (S.J. Suh); same data, except for 27-ix-2005; Choryang-dong, Busan, 9 specimens, on *Pyracantha* sp. (Rosaceae), 29-ix-2006 (S.J. Suh); Uponeup, Changwon, 1 specimen, on *Robinia pseudoacacia* (Fabaceae), 25-vi-2008 (S.W. Park); Oedo, 2 specimens, on *Rhododendron indicum* (Ericaceae), 6-xi-2008 (S.J. Suh); Danmok-ri, Jinju, 5 specimens, unknown plant, 10-i-2011 (S.J. Suh); Jungang-dong, Busan, 3 specimens, on *Camellia japonica* (Theaceae), 6-i-2012 (S.J. Suh); same data, except for on *Rhododendron indicum* (Eri-

caceae). JB: Jeonju-arboretum, 1 specimen, unknown plant, 16-iii-2006 (S.J. Suh); Buan-gun, 2 specimens, *Euonymus japonicus* (Celastraceae), 17-iii-2006 (S.J. Suh). JN: Mokpo, 12 specimens, on *Rhododendron* sp. (Ericaceae), 10-v-2007 (S.J. Suh); Wando-arboretum, 1 specimen, on *Zanthoxylum planispinum* (Rutaceae), 18-vi-2008 (S.J. Suh). JJ: Wolpyeong-dong, Seogwipo, 4 specimens, on *Citrus unshiu* (Rutaceae), 21-iii-1998 (S.M. Oh); Jeju, 1 specimen, on *Morus alba* (Moraceae), 11-v-2006 (S.Y. Gim).

**Hosts.** Polyphagous (fifteen plant families) (Ben-Dov *et al.*, 2012).

**Distribution.** Palaearctic: Korea; China; Japan; Italy; United Kingdom; Canary Islands. Oriental: China; Hong Kong; Indonesia; Cambodia; Malaysia; Philippines; Ryukyu Islands; Sri Lanka; Taiwan; Thailand; Vietnam. Australasian: Australia; Christmas Island; Cook Islands; Fiji; Guam; Hawaiian Islands; New Caledonia; New Zealand; Palau; Papua New Guinea; Tonga; Vanuatu. Afrotropical: Tanzania; Uganda. Nearctic: Mexico; United States of America. Neotropical: Brazil; Chile; Guadeloupe; Jamaica; Panama; Puerto Rico & Vieques Island; U.S. Virgin Islands (Ben-Dov *et al.*, 2012).

**Economic importance.** This species is especially an economic pest of ornamental and garden plants growing in urban. It secretes large quantities of honeydew which provides a medium for sooty mold. The sooty mold may become so dense that it interferes with photosynthesis (Hamon and Williams, 1984).

**Remarks.** We determined that the 143 specimens labeled as *C. pseudoceriferus*, deposited at the National Academy of Agricultural Science, Korea were actually *C. ceriferus*.

## Identification tools

The dichotomous keys of wax tests and slide mounted characters are provided to distinguish adult females of *Ceroplastes* species from Korea.

## Key to the wax tests of adult females *Ceroplastes* in Korea

1. Wax test (or cover) white to pinkish white, round to oval or irregular in dorsal view, with an anteriorly projecting horn (Fig. 1A) ..... *Ceroplastes ceriferus* (Fabricius)

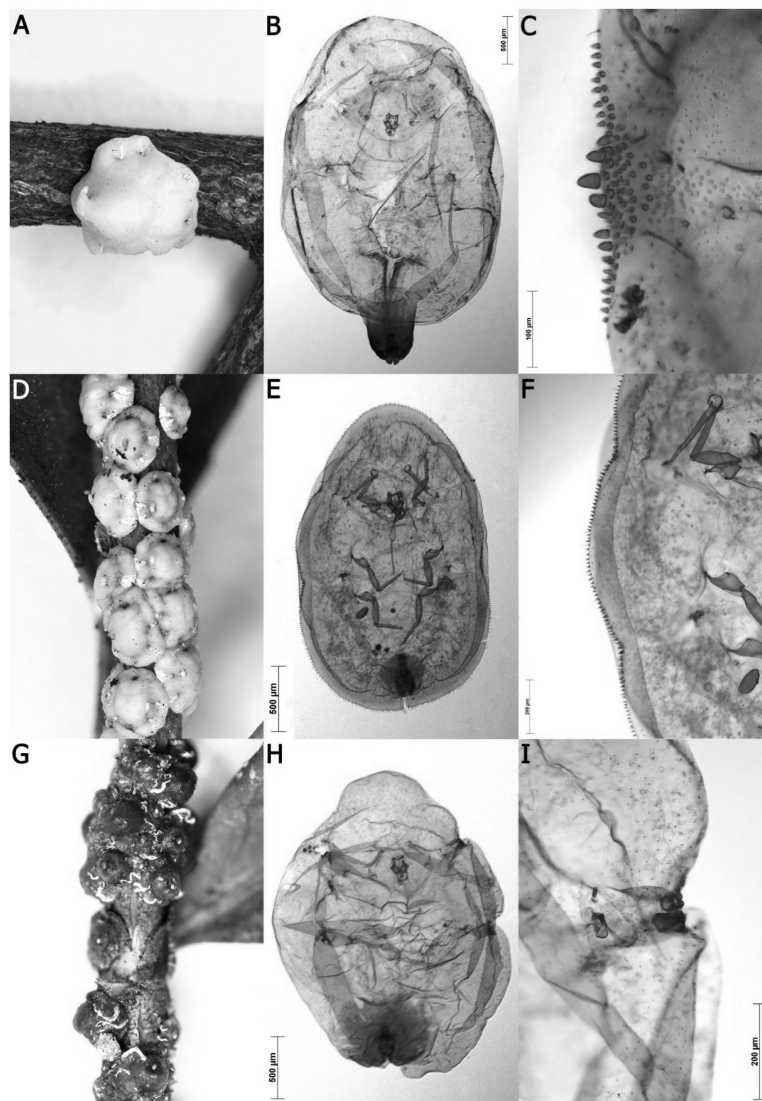
- 1b. Wax cover, beige to grayish white or pink to reddish, pentagonal to hexagonal in dorsal view, without horn (Fig. 1D, G) ..... 2
- 2b. Wax cover pentagonal in dorsal view, pink to reddish (Fig. 1G) ..... ***Ceroplastes rubens* Maskell**
- 2b. Wax cover hexagonal in dorsal view, grayish white (Fig. 1D) ..... ***Ceroplastes japonicus* Green**

**Key to slide mounted adult females *Ceroplastes* in Korea**

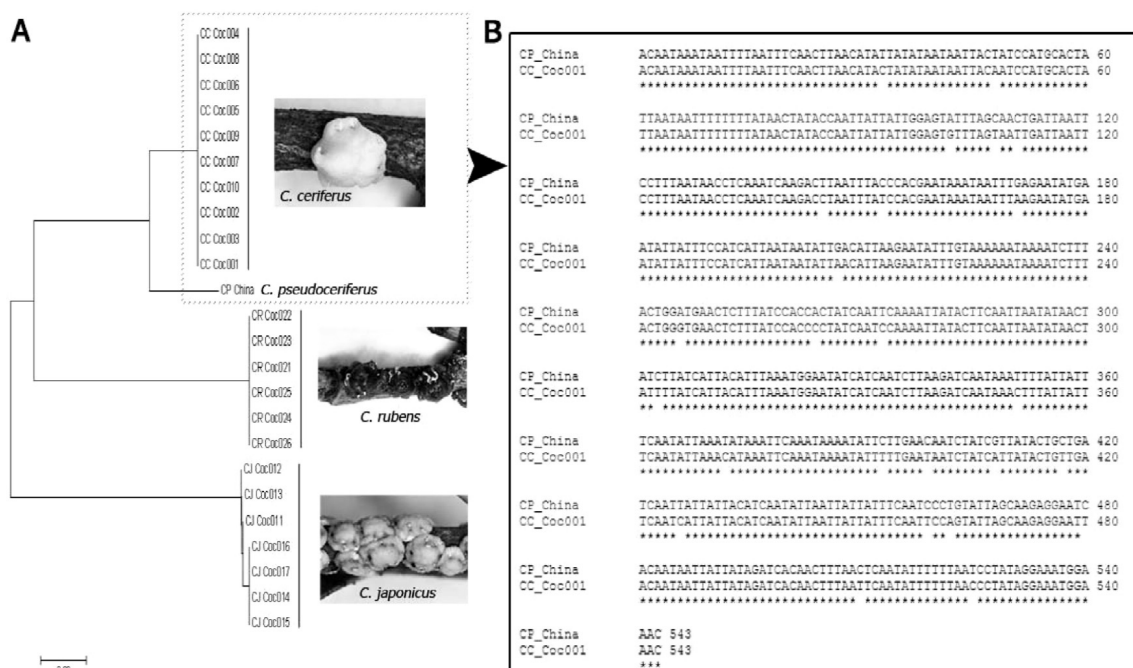
- 1. Mediodorsal clear area present; ventral tubular ducts absent;

- legs reduced (Fig. 1H) ..... ***Ceroplastes rubens* Maskell**
- 1b. Mediodorsal clear area absent; ventral tubular ducts present; legs well developed (Fig. 1B, E) ..... 2
- 2. Stigmatic setae arranged in 6 irregular rows present discontinuously between anterior and posterior stigmatic furrows (Fig. 1C) ..... ***Ceroplastes ceriferus* (Fabricius)**
- 2b. Stigmatic setae arranged in 3 irregular rows present continuously between anterior and posterior stigmatic furrows (Fig. 1F) ..... ***Ceroplastes japonicus* Green**

We consider that *C. pseudoceriferus*, does not exist in Korea, and that previous records of this species in Korea represent a



**Fig. 1.** A-B) *Ceroplastes ceriferus*, adult female; C) *C. ceriferus*, anterior stigmatic setae; D-E) *C. japonicus*, adult female; F) *C. japonicus*, setae between anterior and posterior stigmatic furrow; G-H) *C. rubens*, adult female; I) *C. rubens*, anterior stigmatic setae.



**Fig. 2.** A) COI neighbor-joining tree of *Ceroplastes* species from Korea; B) Alignment of COI barcode region from *C. ceriferus* (CC\_Coc001) and *C. pseudoceriferus* (CP\_China).

misidentification of *C. ceriferus*. *C. pseudoceriferus* most nearly resembles *C. ceriferus*, but can be distinguished from the latter species by following characters: *C. pseudoceriferus* has about 40 marginal bristle-shaped setae between the anterior stigmatic furrows; about 10 such setae between each anterior and posterior stigmatic furrow; and about 130 stigmatic setae laterad of each stigmatic furrow. Whereas, *C. ceriferus* possesses about 10 marginal bristle-shaped setae between the anterior stigmatic furrows; about 3 such setae between each anterior and posterior stigmatic furrow; and about 54 stigmatic setae laterad of each stigmatic furrow (Gimpel *et al.*, 1974; Tang, 1991).

In addition, we used the 543 base pair DNA barcode region of the COI for the discrimination of allied species, *C. ceriferus* and *C. pseudoceriferus*. The alignment of their sequences showed 25 base-pair mismatch positions and the COI divergence was 4.6% (Fig. 2). COI-based sequences were indicative of different species, which also provided genetic evidence to support the separation of *C. ceriferus* from *C. pseudoceriferus* based on morphological differences.

The proper evaluation of the potential threat of a *Ceroplastes* species intercepted on imported plants and the determination of

the appropriate response or action that needs to be taken, require the knowledge of which species of *Ceroplastes* are already known to occur in Korea. We confirmed that three species, *C. ceriferus* (Fabricius), *C. japonicus* Green, and *C. rubens* Maskell, occur in Korea, through carefully re-examination of previously collected specimens and recent survey. The morphology and DNA-based approach can make accurate identification possible. Morphological identification is primarily based on characteristics of the adult female. With the use of DNA, it is now possible to identify some life stages such as eggs and young larvae of *Ceroplastes* species that were previously not identifiable to species level.

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