Polymerase Chain Reaction Identification of a Hymenopteran Insect in the Cornea: A Case Report

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The type of corneal injuries associated with insect encounters is related to the composition of the foreign body. However, previous reports on corneal foreign bodies as insects were rarely based on scientific evidence. Here, we report on a 49-year-old male who was stung in his left eye by an unknown insect. Emergent keratotomy was performed to remove the embedded corneal foreign body. The removed foreign body was observed under light microscopy, and a fragment of insect was suspected. The sample was sent for molecular analysis. The polymerase chain reaction product was sequenced, subjected to a BLAST search, and identified as an ichneumonoid member of the insect order Hymenoptera.

Key Words: corneal insect foreign body, polymerase chain reaction, Hymenoptera
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Corneal injuries have been reported to be associated with various insects and spiders. These have included bee and wasp stings, caterpillar and tarantula hairs, insect wings, fly larvae, and imported fire ants [1–10]. The reaction, either a local inflammation or a potential for permanent visual loss, was related to the composition of the foreign body [8,11]. However, the foreign bodies in the corneas examined were reported previously to be insects mostly on the basis of clinical suspicion and rarely scientific evidence. Lawton reported the first case with corneal insect fragments examined under microscopy in 1988 [11], and Al-Towerki presented the only case with a removed stinger observed under electron microscopy in 2003 [12]. We report a case with an insect sting of the cornea. A retained fragment was surgically removed and analyzed under histologic observation and molecular diagnosis.

Case Presentation

A 49-year-old male was stung in his left eye by an unknown insect while riding a motorcycle on 8 August 2003. The patient immediately experienced pain, tearing, and mild blurred vision. He visited an ophthalmologist 1 hour later, and only the fragment outside the cornea was removed. Unfortunately, the eye pain became more intense and the vision in his left eye deteriorated rapidly. Thus, he came to our hospital 12 hours after the accident.

On initial ocular examination, his best-corrected visual acuity was 0.02 in the left eye and 2.0 in the right eye. Slit lamp biomicroscopy examination of the left eye showed

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marked chemosis, conjunctival injection, and severe corneal edema. A brownish fragment was embedded in the peripheral corneal stroma at the nasal upper quadrant with a ring-shaped infiltration around the foreign body (Figure 1). Hypopyon, 3+ cells, and flare were noted in the anterior chamber. The intraocular pressure was 10 mmHg in his right eye and 11 mmHg in his left eye.

Emergent keratotomy was performed to remove the retained fragments. Postoperatively, he was treated with systemic steroid and antihistamine, as well as local therapies with antibiotics, antihistamine, cycloplegics, and steroids. The condition improved gradually on follow-up examination (Figure 2). The patient's visual acuity in the left eye had improved 2 weeks after the surgery to 0.9. Corneal edema subsided and no inflammation was noticed in the anterior chamber. A circular opacity remained in the area of the cornea surrounding the sting (Figure 2C). No cataract formation or iris atrophy was seen during the follow-up period.

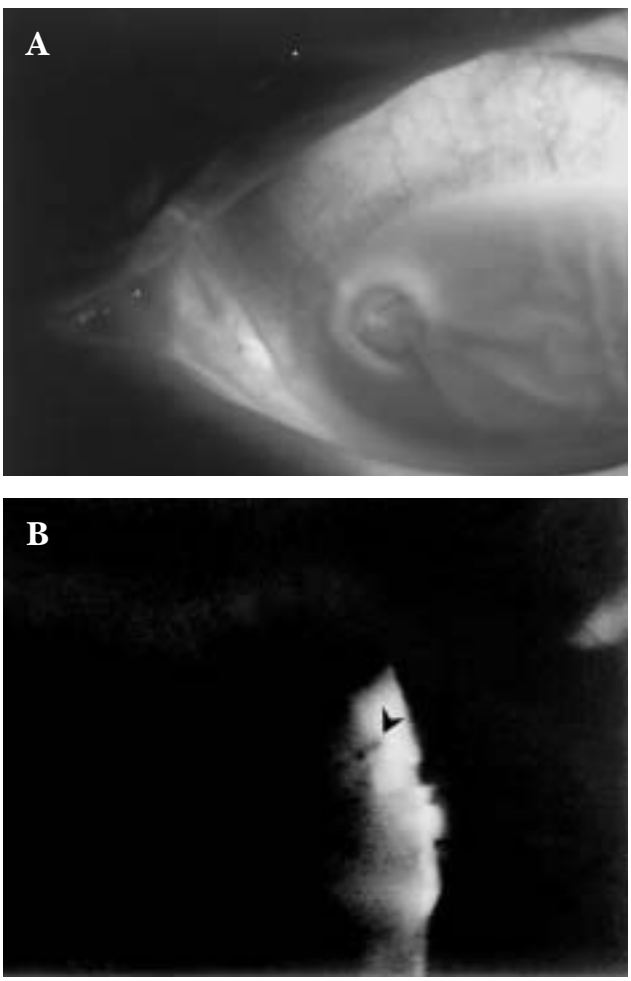

Figure 1. *Severe corneal edema, conjunctival congestion, chemosis, and a ring-shaped infiltration around the foreign body (A). Slit lamp biomicroscopy demonstrated a brown stinger in the corneal stroma (arrowhead) with mild melting presentation (B).*

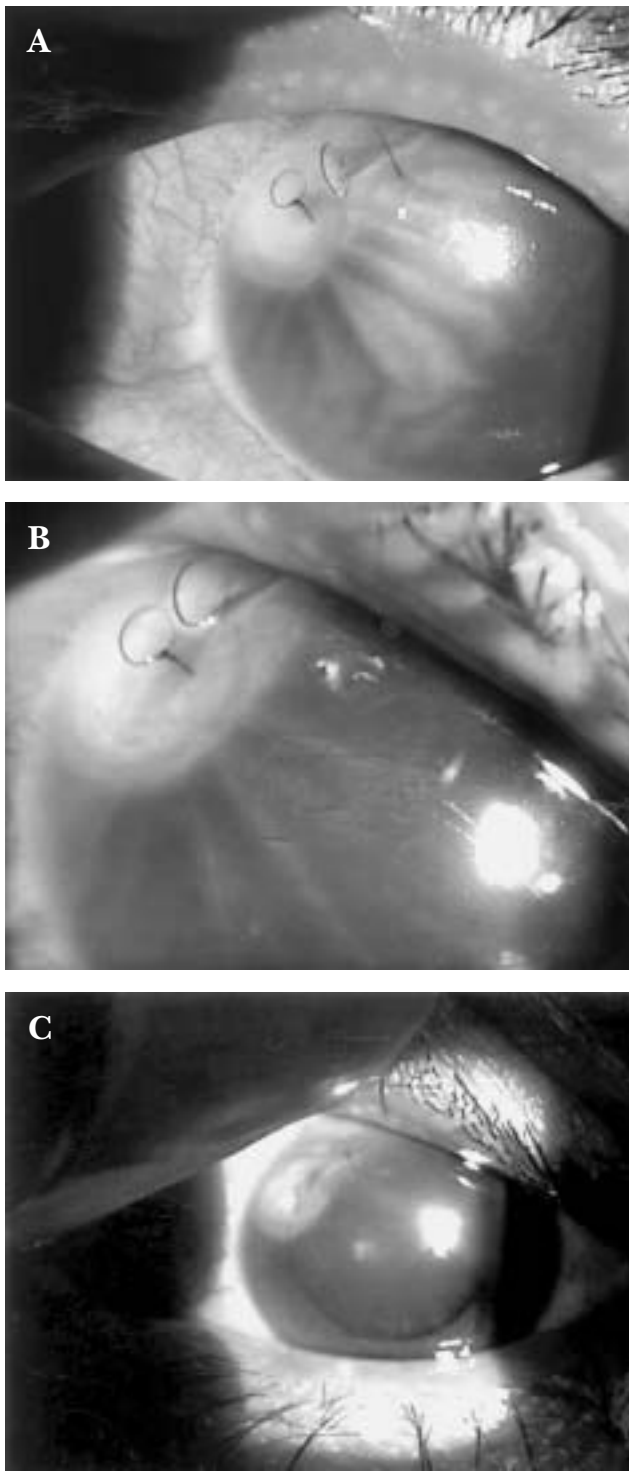

Figure 2. *Cornea edema and infiltration diminished progressively after surgical removal of the stinger. Illustrations show the condition (A) 3 days, (B) 7 days, and (C) 17 days after the operation.*

After removing the embedded fragment, we observed it under a light microscope. Gland-like structures accompanied with chitin-like material were noticed and ascertained to be parts of an insect (Figure 3). The removed foreign body was further sent for molecular analysis by polymerase chain reaction (PCR), using universal primers of the mitochondrial 16S rDNA for insects (Table) [13]. A highly sensitive DNA extraction method was used to extract the miniscule amount of sting DNA [14], and a DNA template that came from another insect (tiger beetle; *Cicindela aurulenta*, Coleoptera) was used as a positive control. The amplified PCR product was about 300 base pairs (bp) in length and was sequenced directly using both amplified PCR primers. The sequence of the products (Figure 4) was used as a query nucleotide to search its homolog sequences (http:/www.kinase.com), and the optimal BLAST result was identified to belong to an insect of the order Hymenoptera. The seven blasting sequences in the most optimal condition were from GenBank and were included in the phylogenetic analysis. The phylogenetic result showed that the sting DNA sequence of the foreign body was a member of the hymenopteran superfamily Ichneumonoidea (Figure 5).

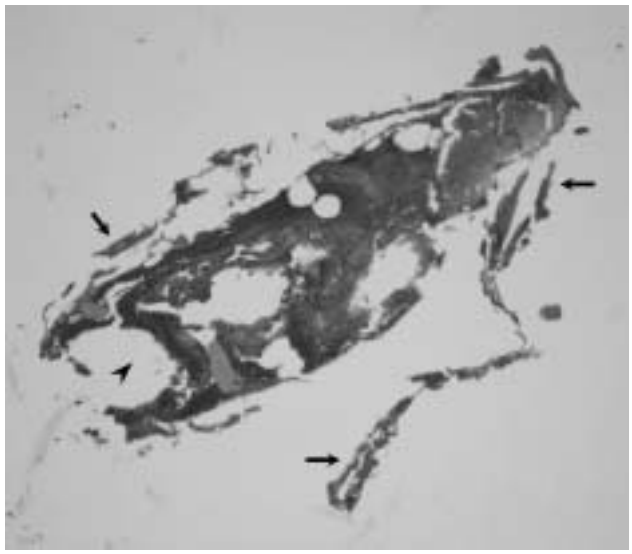

Figure 3. *The fragment is 1.0 mm in length. The arrowhead indicates gland-like structures that are encircled by the chitin-like material (arrow). A noncellular structure with uniform, glossy, and refractive staining could be observed in the chitin-like material. It was fragile during routine pathologic tissue handling and could be considered to be the insect's exoskeleton according to the morphologic characteristics (hematoxylin & eosin, × 100).*

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Query: 2   gacgataagaccctatagaattt 24
           ||||||||||||||||||||||||
Sbjct: 174 gacgataagaccctatagaattt 196

Query: 110 atcctaatatttagataaaaagataaaattaccttagggataacagcataatttttttta 169
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Sbjct: 320 atcctaatatttagattaataaattaaattaccttagggataacagcataatttttttg 379

Query: 170 aaagatcttattaataaaaaagattatgacctcgatgttgaattaaaataaaatttt 226
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 380 aaagttcttattaataaaaaagattatgacctcgatgttgaattaagataaaatttt 436
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Figure 4. *BLAST result of the partial 16S rDNA sequence from sting of unknown insect. The optimal search result is the subject of hymenopteran Oncophanes species (AF 176050).*

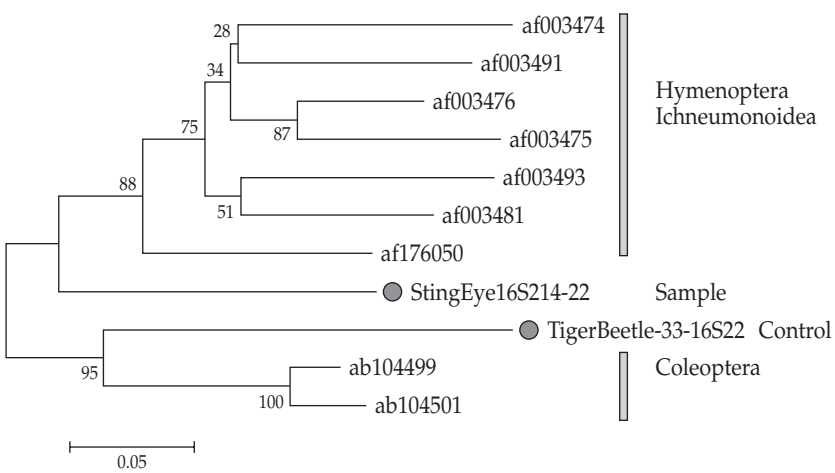

Figure 5. *Phylogenetic analysis of the partial mitochondrial 16S rDNA sequences (290 base pairs) using the neighbor-joining clustering method under the Kimura-2-parameter distance model. Seven sequences in the BLAST search and the referenced sequence from the coleopteran insect are included in this analysis.*

Table. Primer sequences used in this study

Abbreviation of primer	Direction*	Nucleotide sequences
16S214	+	5'-GGACGATAAGACCCT-3'
16S22	–	5'-CCGGTCTGAACTCAGATCA-3'

16S = a partial region of the mitochondrial 16S rRNA gene.
*Upstream and downstream primers are represented by the symbols + and –, respectively.

Discussion

Corneal damage caused by an insect foreign body is rare and has been reported infrequently. The injuries may be due to penetration, or to an immunologic or toxic reaction. They may also result from all three mechanisms combined [15]. Disorders caused are usually limited to the cornea and conjunctiva, such as corneal edema, corneal infiltration, chemosis, and hyperemia of the bulbar conjunctiva. However, other serious ocular complications may result as well, including hypopyon, hyphema, uveitis, lens subluxation, cataract formation, iris depigmentation, and sector iridoplegia [2–4,15,16]. Cases of optic neuritis and papilledema after a bee sting have been reported rarely [17–20].

The composition of the foreign body determines the extent of inflammatory response. For example, bee or wasp stings usually produce acute reactions due to the toxic agents contained in their venom [1,6]. Therefore, identification of the material embedded in the cornea is important. Previous reports of corneal insect foreign bodies were mostly based on clinical suspicion, patients' history, or memory. Only in a few cases have the causative agents of insects been recognized by scientific methods, such as observation under a light microscope or an electron microscope [11,12]

In this case, the severe eye pain after the accident may have been caused by a sudden release of highly concentrated biologic amines, such as histamine. The chemosis and marked conjunctival injection may have resulted from the toxic effects of biogenic amines, which produce vasodilatation and an increase in capillary permeability [4]. However, these phenomena may also have been induced by an immunologic reaction to high-molecular-weight enzymes. Smolin and Wong surmised that high-molecular-weight enzymes in the venom of insects may induce type I hypersensitivity response mediated by immunoglobulin E [2]. Clinically, corneal edema is the hallmark of an anaphylactoid type of immune reaction, and the white corneal infiltration is the result of the chemotaxis of polymorphonuclear leukocytes [4]. The polymorphonuclear leukocytes release their proteolytic enzymes and cause cellular necrosis [2]. In this case, sting stimulation of one species of Hymenoptera was the cause of these reactions.

In summary, this is a case of corneal insect sting. The condition improved significantly after surgical removal of the remaining foreign body. It offers a more scientific way to identify the nature of the insect foreign body. To the best of our knowledge, this is the first report using molecular analysis to identify an insect foreign body embedded in the cornea.

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以聚合酶連鎖反應 (PCR) 確認角膜上之膜翅目昆蟲一病例報告

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昆蟲異物引起的角膜反應與該異物的成份有關。然而，之前報告過的角膜昆蟲異物很少以科學的方法佐證。 我們提出一位 **49** 歲男性，其左眼遭一未知之昆蟲撞擊。經緊急角膜切開術取出異物後，將檢體置於光學顯微鏡下觀察並作分子分析。將聚合酶連鎖反應 (PCR) 之產物作排序並比對，屬於膜翅目 (**Hymenoptera**) 昆蟲姬蜂總科的近緣物種。

關鍵詞：角膜昆蟲異物，聚合酶連鎖反應，膜翅目

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