RINGKASAN

Ambergris merupakan substansi langka yang terbentuk dalam sistem pencernaan Paus Spermaseti (*Physeter macrocephalus* L.), Paus Spermaseti Kerdil (*Kogia breviceps*), dan Paus Spermaseti Cebol (*Kogia sima*). Material *ambergris* yang setelah mengapung di laut dalam waktu lama berpotensi sebagai sampel untuk analisis DNA purba (*ancient* DNA/aDNA). Penelitian ini bertujuan untuk menentukan protokol isolasi dan amplifikasi DNA dari sampel *ambergris* yang telah terdegradasi akibat paparan lingkungan laut, serta mengevaluasi efektivitas marka gen mitokondria *Cytochrome c oxidase subunit I* (COI) dan *Cytochrome b* (CytB) dalam proses identifikasi molekuler spesies Paus penghasil *ambergris*. Sampel *ambergris* (CLP-01 dan CLP-02) yang ditemukan di perairan Cilacap, Jawa Tengah, dianalisis menggunakan empat metode isolasi DNA: Chelex 100® 5%, fenol-kloroform, kit komersial berbasis kolom silika, dan teknik *boil-preparation*. Kuantitas DNA diukur menggunakan spektrofotometri UV-Vis Nanodrop, sedangkan amplifikasi gen COI dan CytB dilakukan melalui PCR dengan beberapa optimasi kondisi, termasuk penambahan BSA 1% dan DMSO 1-5% untuk meminimalkan efek dari senyawa inhibitor pada PCR dan amplifikasi menggunakan Phusion (*Pfu*) DNA *Polymerase*.

Hasil isolasi DNA menunjukkan bahwa hanya metode Chelex® 5% yang berhasil mengisolasi DNA dari salah satu sampel, yakni CLP-01 dengan konsentrasi 181 ng/µL, meskipun menunjukkan indikasi kontaminasi protein dan kemungkinan terbentuknya senyawa Maillard. Hasil seluruh optimasi PCR tidak berhasil menghasilkan amplikon spesifik baik untuk marka gen COI maupun CytB, mengindikasikan adanya degradasi materi genetik serta perubahan struktur materi genetik pada sampel sehingga menghambat proses amplifikasi. Selain itu, analisis terhadap salah satu sampel, CLP-02, menunjukkan karakteristik yang tidak sesuai dengan *ambergris* asli, melainkan lebih menyerupai *spermaceti wax* atau residu minyak sawit (*palm oil residue*), sehingga tidak mengandung DNA spesies paus penghasil yang dapat dianalisis lebih lanjut.

Penelitian ini menguraikan berbagai kendala teknis dalam isolasi dan analisis DNA dari material *ambergris*, terutama akibat tingginya kandungan lipid, degradasi DNA, serta potensi reaksi kimia seperti pembentukan senyawa Maillard. Meskipun analisis PCR belum berhasil mendeteksi spesies paus penghasil *ambergris* secara pasti, studi ini diharapkan dapat menjadi protokol awal untuk karakterisasi molekuler sampel *ambergris* di Indonesia. Temuan ini diharapkan dapat menjadi dasar bagi pengembangan studi DNA purba (aDNA) dan penelitian paleogenomik di wilayah tropis, khususnya untuk material biologis terdegradasi dari lingkungan laut.

Kata kunci: *Ambergris, Chelex 100®, DNA purba, DNA terdegradasi, Identifikasi molekuler, Paleogenomik, PCR, Reaksi Maillard, Cytochrome b (CytB), Cytochrome c oxidase subunit I (COI)*

SUMMARY

Ambergris is a rare substance formed in the digestive systems of sperm whales (*Physeter macrocephalus* L.), pygmy sperm whales (*Kogia breviceps*), and dwarf sperm whales (*Kogia sima*). Ambergris material that has floated at sea for extended periods holds potential as samples for ancient DNA (aDNA) analysis. This study aimed to determine a protocol for the isolation and amplification of DNA from ambergris samples that have undergone degradation due to prolonged exposure to marine environmental conditions, as well as to evaluate the effectiveness of mitochondrial gene markers *Cytochrome c oxidase subunit I (COI)* and *Cytochrome b (CytB)* in the molecular identification of whale species associated with ambergris origin. Two ambergris samples (CLP-01 and CLP-02), collected from the coastal waters of Cilacap, Central Java, Indonesia, were analyzed using four DNA isolation methods: Chelex 100® 5%, phenol-chloroform extraction, a silica column-based commercial kit, and the boil-preparation technique. DNA quantity was measured using UV-Vis Nanodrop spectrophotometry, while COI and CytB gene amplification was conducted through PCR with several condition optimizations, including the addition of 1% BSA and 1-5% DMSO to minimize PCR inhibitor effects, and amplification using Phusion (Pfu) DNA Polymerase.

The DNA isolation results demonstrated that only the Chelex® 5% method was successful in recovering DNA from one of the samples, CLP-01, with a concentration of 181 ng/µL, although spectrophotometric analysis indicated potential protein contamination and the presence of Maillard reaction products. Despite multiple optimization strategies, including the use of PCR additives and high-fidelity polymerases, no specific amplicons were obtained for either the COI or CytB gene markers. This suggests significant degradation and possible structural modification of the genetic material, which likely impeded primer binding and amplification. Additionally, the second sample, CLP-02, exhibited physical and chemical characteristics inconsistent with authentic ambergris, resembling instead spermaceti wax or palm oil residue, and did not contain detectable whale DNA suitable for molecular identification.

This study outlines various technical challenges in DNA isolation and analysis from ambergris material, primarily due to high lipid content, DNA degradation, and potential chemical reactions like Maillard compound formation. Although PCR analysis did not conclusively detect the whale species producing the ambergris, this study establishes a preliminary protocol for molecular characterization of ambergris samples in Indonesia. These findings may serve as a foundation for developing ancient DNA (aDNA) studies and paleogenomic research in tropical regions, particularly for degraded biological materials from marine environments.

Keywords: Ambergris, Chelex 100®, Ancient DNA, Degraded DNA, Molecular identification, Paleogenomics, PCR, Maillard reaction, Cytochrome b (CytB), Cytochrome c oxidase subunit I (COI)