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# Using DNA-barcoding to make the necrobiont beetle family Cholevidae accessible for forensic entomology

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ABSTRACT

The beetle family Cholevidae (Coleoptera: Staphylinoidea), sometimes viewed as the subfamily Cholevinae of the Leiodidae, consists of some 1700 species worldwide. With the exception of specialized cave-dwelling species and species living in bird and mammal nests and burrows, the species are generalized soil-dwellers that, at least in temperate regions, are mostly found on vertebrate cadavers. Although they have been regularly reported from human corpses, and offer potential because of many species' peak activity in the cold season, they have not been a focus of forensic entomologists so far. This is probably due to their small size and the difficulty in identifying the adults and their larvae. In this paper, we show that DNA-barcoding can help make this group of necrobiont beetles available as a tool for forensic research. We collected 86 specimens of 20 species of the genera *Catops*, *Fissocatops*, *Apocatops*, *Choleva*, *Nargus*, *Ptomaphagus*, and *Sciodrepoides* from the Netherlands and France and show that a broad "barcoding gap" allows almost all species to be easily and unambiguously identified by the sequence of the "barcoding gene" cytochrome *c* oxidase I (COI). This opens up the possibility of adding Cholevidae to the set of insect taxa routinely used in forensic entomology.



## 1. Introduction

Exposed vertebrate cadavers, including human ones, form a transient, but nutrient-rich food source that is exploited by many groups of invertebrate animals, especially insects. Numerous ecological studies (e.g., [1–5]) have investigated the abundance, species diversity, succession, and ecological interactions of insects on decaying cadavers. These studies have shown that the ecological preferences of many species are very narrowly circumscribed and that the insect community changes continually and in predictable ways. Hence, the composition of species and in particular their life-stages on a corpse allows forensic entomologists to make accurate pronouncements on the Postmortem Interval (PMI) and on other events surrounding the body since death [6]. Despite the increased popularity of forensic entomology, the potential of the insect assemblages on corpses has not been completely tapped. For example, although the carrion-inhabiting fauna includes members from a wide variety of arthropod taxa (e.g., Diptera, Coleoptera, Hymenoptera, Lepidoptera, and Acari [7]), cases where entomological evidence contributes to death investigations almost exclusively involve Diptera [8,9]. Coleoptera, for example, are much less popular, despite the fact that they are taxonomically and ecologically more diverse than Diptera. Among the Coleoptera inhabiting corpses are scavengers (e.g., Ptiliidae, Dermestidae) as well as predators (e.g., Histeridae and certain Silphidae), and groups that are specialized on early (e.g., *Nicrophorus* silphids), middle (e.g., many Staphylinidae), and late (e.g., Dermestidae) stages of decomposition [5,8,9]. Thus, potentially Coleoptera include species that could yield forensic information complementary to that obtained from Diptera. The reasons for the relative rarity of Coleoptera and many other necrobiont insect groups in forensic entomological practice probably include their taxonomic inaccessibility, especially where species-rich and small-bodied groups are concerned [10].

One such group is the family Cholevidae. These staphylinoid beetles, sometimes viewed as a subfamily (Cholevinae) of the

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Leiodidae, comprise some 1700 species world-wide [11]. All are small (mostly 2–6 mm in length), ovoid in body shape, brown, grey or black, and as adults distinguishable only by subtle differences in the proportions of antennal articles, pronotal shape, and the genitalia [12]; identification of the larvae is equally cumbersome (Zwick, personal communication). Although they include some groups that are highly derived cave-dwellers (such as the subfamily Leptodirinae) or specialised inhabitants of mammal nests and burrows (e.g., the genus *Choleva*), the majority of the species are found above-ground on decaying matter, most commonly animal cadavers, where they probably feed predominantly on fungal spores [13,14]. In temperate regions, they are among the dominant insect groups found on animal carcasses, both above-ground [5] and buried [15]. E.g., Kočárek [16] collected 586 specimens on beef heart in the Czech Republic, making them (in that study) the third most numerous beetle family after Silphidae and Staphylinidae. Also in the Czech Republic, Růžička [17] collected 8903 cholevid specimens on rotten fish, by which they were as common as silphid burying and carrion beetles, and in a Polish study [18], a cholevid species was by far the commonest on dead fish bait, almost twice as common as the next-ranking beetle, and overall, cholevids made up about one-quarter of the total (29,088 individuals). Cholevid larvae are also regularly found on human corpses. For example, Easton and Smith [19] found *Catops tristis* on a human corpse; Lefèbvre and Gaudry [20] in reviewing French forensic entomology cases, report four cases where Cholevidae were collected, although it is likely that they were reported under the old family name Silphidae in other cases; the Alaskan species *Catoptrichus frankenhaeuseri* was even first discovered on a human cadaver, in 1852 [21]; and forensic entomological specimens collected in the Netherlands during police investigations also regularly include Cholevidae: out of 25 cases with coleopterans present, 20% (all of which collected during winter in rural settings) contained cholevid larvae (J. Huijbregts, personal communication). Nevertheless, they do not figure prominently in the forensic literature, which is all the more surprising in view of their phenology: many species reproduce in autumn and complete their larval development during winter [22]. This means that cholevids are among the few insects that feed on corpses during the cold season, when most dipteran groups are absent, and hence could be a valuable tool for determining PMI in cold-season death investigations.

To help stimulate the use of Cholevidae in forensic investigations in the Netherlands, we have begun compiling a database on distribution, niche preferences, life cycle, and identification characteristics for the Dutch species. Because correct identification of both larvae and adults requires investigation of minute and often internal morphological characters by a specialist, we here report on the use of DNA-barcodes for the identification of Dutch cholevid species. Animal DNA-barcoding relies on the property of animal mitochondrial DNA (mtDNA) variation to coalesce rapidly during evolution [23]; much more rapidly, usually, than the time required for the evolutionary splitting of species. This means that mtDNA variants tend to be contained within species and are usually not shared between species [24]. This "barcoding gap" allows mtDNA sequences to be used reliably for the identification of species [25]. The gene of choice for DNA-barcoding is *cytochrome c oxidase I* (COI), and although problems remain [25–28], DNA-barcoding has been shown to be successful for accurate species-level identifications of insects [29,30], including those of forensic importance [31]. Here, we report on a first attempt to generate reliable DNA-barcodes for Dutch Cholevidae. We find that sequencing a 600-bp fragment of COI from 86 individuals allows them to be sorted quite accurately into 20 recognised, but morphologically very similar species.

## 2. Materials and methods

### 2.1. Trapping and preparing specimens

We used pitfall traps [32] to collect live specimens of Cholevidae. Glass jars were filled with a 3-cm-thick sand layer on which either a piece of chicken or a piece of Limburg or Münster cheese (or both) was placed. These cheese types are common substitutes for decaying meat [33], since they exude a similar mixture of volatiles, and can be used instead of meat if time in the field is limited and it would take too long for meat to have reached the appropriate stage of decay (especially during cold weather). The jar was covered with an iron lid into which 20–30 holes of 6 mm diameter had been punched (to prevent larger animals from accessing the bait) and buried in the soil to such a depth that the ground level was flush with the top of the lid. A plastic roof was placed 10 cm above the trap to prevent rain water from entering. Five traps were placed at each of nine localities in the Netherlands: oak forest on sandy soil at "Noordberg", Heelsum, Prov. Gelderland (51.58°N, 5.44°E); pine forest on sandy soil at "De Sysselt", Ede, Prov. Gelderland (52.02°N, 5.40°E); alder-willow woods on clay-peat soil in the polders west of IJsselstein, Prov. Utrecht

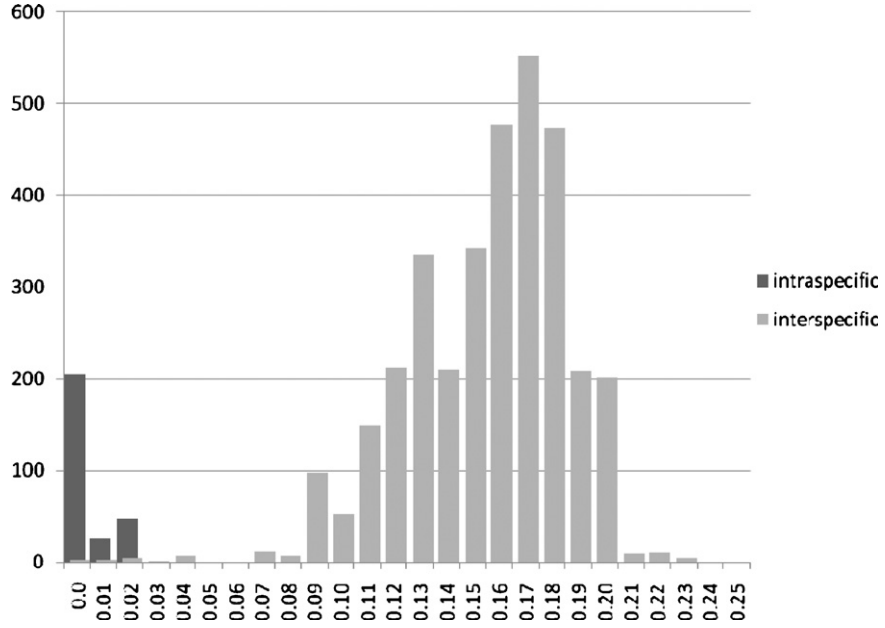


**Fig. 1.** Distribution of pairwise Kimura 2-parameter genetic distances between COI-sequences, revealing a distinct "barcoding gap".