# ARTICLE



# *Adalia decempunctata* (Coleoptera: Coccinellidae), a Palaearctic species now established in North America

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#### Abstract

The Palaearctic ten-spotted lady beetle, *Adalia decempunctata* (Linnaeus), has been confirmed as established in North America, based on morphological characters and DNA barcodes. Its distribution currently appears limited to the Avalon Peninsula of the island of Newfoundland, Canada. Several characters, including the colour of the mesepimera, frons, labrum, antennal club, mouthparts, and legs, are reliable for discriminating among this species and the native, Holarctic two-spotted lady beetle, *Adalia bipunctata* (Linnaeus), in Newfoundland. An identification key to the two species is provided.

# Introduction

The lady beetles (ladybird beetles, ladybugs) are one of the most iconic families of insects in North America, the subject of countless photos, poems, and lore. Consequently, this is one of the best-known families of insects, especially the species in the tribe Coccinellini, which contains the larger and more colourful varieties. There are more than 450 species of lady beetles in North America (Gordon 1985). Brunke et al. (2019) reported 162 species for Canada, and Langor (2019) added one additional species. The Canadian ladybug fauna includes 10 nonnative species, most of which were inadvertently introduced. One notable exception, the harlequin lady beetle, Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae), was intentionally introduced to North America multiple times, starting in 1916, as a biocontrol agent for aphids in greenhouses, subsequently establishing in the wild in the United States of America in 1988 (Chapin and Brou 1991; Hoebeke and Wheeler 1996) and spreading to natural ecosystems in Canada by 1994 (Corderre et al. 1995). Although the seven-spotted lady beetle, Coccinella septempunctata Linnaeus (Coleoptera: Coccinellidae), was also intentionally introduced to North America as a biocontrol agent on numerous occasions between 1956 and 1971, it did not establish as a result of these introductions (Gordon 1985). The first established population of C. septempunctata in North America, found in Bergen Co., New Jersey, United States of America, in 1973, was the result of an accidental introduction (Gordon 1985). Nonnative species are often the most common species encountered in many natural habitats, especially C. septempunctata and H. axyridis.

During examination of undetermined specimens of Coccinellidae from the Canadian province of Newfoundland and Labrador between 2015 and 2018, specimens of *Adalia* with unusual elytral colour patterns were encountered. These were thought to represent colour morphs of the

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Palaearctic species, the ten-spotted lady beetle, A. decempunctata (Linnaeus) (Coleoptera: Coccinellidae), which has an elytral colour pattern that is highly variable throughout its native range (Pope 1953). Furthermore, the putative A. decempunctata specimens possessed pale mesepimera, a key diagnostic character for this species in its native Palaearctic range, whereas mesepimera of the sympatric A. bipunctata (Linnaeus) are typically black (Pope 1953). However, as A. bipunctata populations elsewhere in North America include specimens exhibiting colour morphs similar to those reported for A. decempunctata, and a small proportion of specimens also exhibit paleness of the mesepimera, it was thought prudent to do a detailed morphological and molecular study of the specimens to gather additional evidence to support its identity and to find characters to discriminate among the two species of Adalia. Subsequently, fresh specimens of A. decempunctata were hand-collected from vegetation from the island of Newfoundland for study and preserved as pinned specimens or in 95% ethanol; some of the collected specimens were DNA barcoded. Furthermore, an extensive survey of the Coccinellidae of the island of Newfoundland was conducted in August 2022 and August 2023 (Langor, unpublished data) to, in part, ascertain the distribution of the putative A. decempunctata. We report herein the results of these morphological and molecular studies and provide information on the distribution of this species in North America.

# Materials and methods

## **Collection codons**

Specimens of putative *A. decempunctata* and *A. bipunctata* were examined from the following insect collections:

**BHC** – Barry Hicks Collection, Carbonear, Newfoundland and Labrador, Canada [*A. decempunctata*]. Note: Within about two years, this collection will be transferred to either The Rooms (Newfoundland and Labrador Provincial Museum) or Agriculture and Agri-Food Canada, both in St. John's, Newfoundland and Labrador, Canada.

**CNC** – Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada. [*A. decempunctata* specimens were donated by the senior author.]

**NLC** – Newfoundland and Labrador Collection, The Rooms, St. John's, Newfoundland and Labrador [*A. bipunctata, A. decempunctata*]. This collection consists of most insect material from the former Memorial University of Newfoundland (MUN) Collection, most material from the collection of David Langor, and material from Newfoundland donated by other collectors. The MUN collection was shipped to David Langor Canadian Forest Service, Northern Forestry Centre, Edmonton, Alberta, Canada for safe keeping when the last curator (David Larson) retired in 2005 because the institution no longer wished to retain the collection. From 2023 to 2025, this collection will be relocated to The Rooms (the provincial museum of Newfoundland and Labrador), located in St. John's. It is expected the specimens of Coccinellidae will be transferred in 2023.

**NFRC** – Canadian Forest Service, Northern Forestry Centre, Edmonton, Alberta, Canada. [*A. bipunctata* and *A. decempunctata* specimens were donated by the senior author.]

**UASM** – Strickland Entomological Museum, Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada. [*A. decempunctata* specimens were donated by the senior author.]

#### **Morphological studies**

For morphological studies, 52 specimens of *A. bipunctata* and 24 of *A. decempunctata* from Newfoundland and Labrador were examined using stereoscopic microscopy. Dirty specimens were first cleaned by sonication in soapy water for 15 minutes, followed by a rinse in distilled water and immersion in 95% ethanol for two minutes before being either preserved in 70% ethanol or

pinned. Following cleaning, two specimens of each sex were disarticulated (head, prothorax, meso/meta thoraces, abdomen, legs, wings, elytra), and body parts were closely examined from all aspects to locate diagnostic characters. The genitalia were dissected and examined for two males and females of each species. Body parts and habitus photos were taken using a Leica M80 stereoscopic microscope with a Leica EC3 camera (Leica, Wetzlar, Germany) attached.

#### **DNA studies**

For DNA barcoding, initially five specimens of putative A. decempunctata were chosen from among specimens that had been dried and pinned between 1995 and 2001 and represented several elytral colour morphs. Because these specimens posed challenges for obtaining sequence for the entire Folmer barcoding region of the cytochrome c oxidase 1 (CO1) gene of mitochondrial DNA using existing primers, additional three specimens collected in 2020 were later amplified using polymerase chain reaction and sequenced. Also, specimens of A. bipunctata from Newfoundland (three from 2021) and Alberta (one from 2020) were barcoded. A leg was removed from each specimen, and DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer specifications, with minor modifications during DNA elution, which was performed in multiple rounds of 50 uL to maximise drawdown. The Folmer region (5P) of CO1 was amplified using two different overlapping pairs of primers. The outside primers were CLepFolF (5'-ATT CAA CCA ATC ATA AAG ATA TTG G-3') and CLepFolR (5'-TAA ACT TCT GGA TGT CCA AAA AAT CA-3'). In addition, to improve amplification of the 3' end of the DNA barcode region, a pair of custom internal primers was constructed based on publicly available A. decempunctata sequence data. The primers were designated FolIntDecF (5'-CCA GAT ATG GGC ATT TCC ACG TC-3') and FolIntR (5'-GGG TGG ATA TAC TGT TCA TCC TG-3').

After amplification, DNA was first visualised on a 1% agarose gel and then sequenced at the Molecular Biology Services Unit at the University of Alberta (Edmonton, Alberta, Canada). Sequence data were aligned using the Multiple Alignment using Fast Fourier Transform programme and manually checked in Geneious Prime, version 2020.0.5 (Kearse *et al.* 2012; Katoh *et al.* 2022). Specimens with good-quality sequences for both the 5' and 3' fragments were joined and trimmed to 658 bp. Other databased barcode sequences from *A. bipunctata* (19 specimens) and *A. decempunctata* (23) were retrieved from GenBank, as were three sequences for *Anatis mali* (Say) (Coleoptera: Coccinellidae), which were used as an outgroup.

#### **Distribution survey**

To determine the current distribution of A. decempunctata on the island of Newfoundland, a survey was conducted in August 2022 and in August 2023. The survey method consisted of a 15-minute timed sweep of vegetation at each locality using an 18-inch (46-cm) diameter insect net with a 3-foot (91-cm) handle. The different strata and major groups of vegetation (i.e., trees, shrubs, forbs, grasses, ferns) were swept at each locality in rough proportion to their relative abundance. About 0.1–0.2 ha of vegetation was swept over a 15-minute period. Sampling occurred on rain-free days with air temperatures above 15 °C and with average daytime wind speed below 40 kph. General sample localities were mostly preselected to cover as much of the island of Newfoundland as possible, but specific sites were selected upon arrival, considering accessibility, exposure to wind, and the presence of a wide variety of plants. Rain or high wind prevented some localities from being sampled, and when time allowed, additional localities were sampled. The contents of the sweep net were emptied into a large white plastic pan, and the number of adults of each lady beetle species counted and released, except that all A. decempunctata and a few A. bipunctata were saved in 95% ethanol for future study. Geographic coordinates were determined with a GPS for each site. All specimens of A. decempunctata were databased, as were specimens in various insect collections and online records.

#### **Results and discussion**

#### Morphology

There appears to be no published comprehensive study from the Palaearctic region comparing the morphology of *A. decempunctata* and *A. bipunctata* in detail. A morphological examination of specimens of both species from Newfoundland revealed several characteristics that are of use for discriminating among the two species (Table 1). In contrast to elsewhere in its native range, in Newfoundland, *A. bipunctata* has very low variation in elytral colour patterns. For 50 of 52 specimens studied, the elytron base colour is yellow–orange to orange–brown with a central black spot, which may be partially or completely divided into two smaller spots (Fig. 1B–D). One specimen from Labrador had no evident black spots (Fig. 1A), and one specimen from Labrador had no evident black spots (Fig. 1A), and one specimen from Labrador had no evident black spots (Fig. 1A), and one specimen from Labrador had no evident black spots (Fig. 1A), and one specimen from Labrador had no evident black spots (Fig. 1A), and one specimen from Labrador had two transverse black bands (Fig. 1E). Any other colour morph of *Adalia* in Newfoundland and Labrador should be suspected to be *A. decempunctata* and examined for other character states (Table 1). Eleven colour pattern morphs of *A. decempunctata* have been documented thus far in Newfoundland (Fig. 1F–P), ranging from orange with faint spots on the elytra and pronotum (Fig. 1F) to black pronotum and elytra with an orange patch in the anterior half of each elytron (Fig. 1N). One variant (Fig. 1I) looks somewhat like an *A. bipunctata* than in *A. bipunctata*.

The head and ventral aspect of *A. decempunctata* is typically much lighter in colour compared to *A. bipunctata*, as evident with the antennal club, labrum, mouthparts, gular area, propleura, mesepimera, abdominal sternites, and legs (Fig. 2). These structures range from pale yellow to orange–brown in *A. decempunctata* to black, partially black, or near black in *A. bipunctata* (Table 1). Diagnostic characters used to discriminate between these two species in European populations are as follows: the colour of the mesepimera (*A. decempunctata*: pale and contrasting in colour with adjacent sclerites; *A. bipunctata*: concolorous with adjacent sclerites, black to blackish on almost all specimens observed); anterior margin of mesosternum between coxae (*A. decempunctata*: slightly emarginate; *A. bipunctata*: entire); and external border of abdominal plates (*A. decempunctata*: slightly emarginate; *A. bipunctata*: not, or scarcely, sinuate; Pope 1953; Bienkowski 2018). Among these, the only character useful for discriminating between the two species in Newfoundland is the colour of the mesepimera. Bienkowski (2018) observed that *A. decempunctata* has a transverse fold at the apical declivity that is absent in *A. bipunctata* specimens from Newfoundland. Examination of genitalia revealed no diagnostic characters, which concurs with the findings of Salehi *et al.* (2011).

If A. decempunctata becomes established on the Canadian mainland, it may spread widely, so it is important to know whether the character states used to discriminate among the two Adalia species in Newfoundland will be useful in other parts of the range of A. bipunctata in North America, especially where there is high variation in elytral colour patterns. Specimens of adult A. bipunctata, including colour morphs other than those found in Newfoundland, were examined from most Canadian provinces, the northeastern United States of America, and from Washington state to California, United States of America. The elytral colour morphs of those specimens range from yellow-orange with no spots to orange-brown with two transverse black bands to black with one reddish-orange spot on each elytron. About 300 specimens of A. bipunctata were examined, mostly in the UASM and NFRC collections, including about 210 specimens of the common 'one black spot per elytron' variety typical of Newfoundland populations and 87 of other colour morphs. All but 13 of these specimens had black mesepimera that were concolorous (or nearly so) with adjacent sclerites. Thus, 95.6% of A. bipunctata from this sample can be separated from A. decempunctata by mesepimera colour alone. Of the 13 specimens of A. bipunctata with light mesepimera (seven from Alberta, Canada, five from New Jersey, United States of America, and one from Minnesota, United States of America), 12 were readily distinguishable from A. decempunctata by the presence of a medial black area on the frons (between the eyes). The remaining specimen (Medicine Hat, Alberta) had black legs, dark antennal club, and dark mouthparts, all character states that have not been seen

Adalia bipunctata	Adalia decempunctata	
a. Mesepimera completely black in more than 95% of specimens, concolorous with adjacent sclerites	a'. Mesepimera mostly or completely milky white to yellowish orange, contrasting with adjacent black sclerites	
b. Frons between eyes with black central area, which typically covers $\frac{1}{2}$ to $\frac{1}{2}$ of the distance between the eyes, but sometimes it is very narrow; areas of frons adjacent to eyes yellowish brown	b'. Frons between eyes entirely yellowish brown, rarely with central black area (seen only in two specimens with black elytra a single orange spot on each elytron (Fig. 2P))	
c. Labrum black or blackish, darker than the pale areas on the frons.	c'. Labrum pale yellow to brown, about same colour as tibiae and tarsi.	
d. Legs black (or mostly so), about same colour as meso- and metathoracic sternites	d'. Legs (or at least tibia and tarsi) yellow-orange to orange-brown, paler than meso- and metathoracic sternites	
e. Antennal club darker than yellowish orange to orange–brown flagellum	e'. Antennal club same colour as flagellum, both yellowish to orange brown, although sometimes the distal antennomere is darker than the others	
f. Maxillary palpi with terminal segment darker (blackish), at least in distal half, than other segments (yellowish)	f'. Maxillary palpi entirely milky white to yellowish, except some specimens have a narrow dark fringe on the tip of the distal segment	
g. Labial palpi with all segments blackish	g'. Labial palpi entirely milky white to yellowish orange	
h. Elytra base colour yellow orange to orange brown with large to small central black spot, which may be partially or completely divided into two smaller spots (Fig. 1B–D) or no evident spots (Fig. 1A; rare). One specimen from Labrador has two transverse, black elytral bands, one at the midpoint and one at the posterior of the elytra (Fig. 1E). There is little variation in colour patterns in Newfoundland and Labrador in contrast to elsewhere in its range	h'. Elytra colour ranges from black with orange spots to yellowish orange with 0–10 spots of various sizes (Fig. 1F–P)	

# Table 1. Summary of morphological characters useful for discriminating among Adalia bipunctata (Linnaeus) and A. decempunctata (Linnaeus) in Newfoundland.

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Figure 1. Dorsal habitus photos for colour variants of *Adalia bipunctata* (Linnaeus) and *Adalia decempunctata* (Linnaeus) in Newfoundland and Labrador; **A-E**, *A. bipunctata*; **F-P**, *A. decempunctata*.

to date in *A. decempunctata* in North America. Thus, it appears the suite of characters useful for discriminating among these two *Adalia* species in Newfoundland will also be useful elsewhere in Canada. Care must be taken with teneral specimens because the venter, including mesepimera, may be pale, but in these cases, the pale mesepimera are concolorous with adjacent sclerites.



Figure 2. Photos of morphological structures and character states for two species of *Adalia* in Newfoundland: A, A. bipunctata labrum, B, mesothorax, and C, ventral habitus; D, A. decempunctata labrum, E, mesothorax, and F, ventral habitus.

# A key to separate the two Adalia species in North America

#### DNA

It was not possible to sequence the entire Folmer barcoding area of CO1 for the older specimens of pinned *A. decempunctata* using existing primers that have worked for *Adalia* elsewhere, likely indicating that the mitochondrial DNA was somewhat denatured. Interestingly, the 5' end of the barcode region did amplify well for these specimens, but the 3' end did not. The three fresh specimens of putative *A. decempunctata* from Newfoundland amplified well, and DNA sequence from the barcode region confirms the identity as *A. decempunctata* (Fig. 3; Table 2). In addition, the four fresh specimens of *A. bipunctata* amplified, and the DNA sequences confirm their identities. These sequences were submitted to the Barcode of Life Datasystems (BOLD) database (https://www.boldsystems.org; Table 2).

#### **Distribution and spread**

All collection localities for *A. decempunctata* in Newfoundland are on the northern third of the Avalon Peninsula, ranging from St. John's (the site of the earliest record, 1995) along the shore of Conception Bay to as far north as Carbonear. During a survey of Coccinellidae of Newfoundland in 2022 and 2023, no specimens were found at eight sampling sites further south on the Avalon Peninsula or elsewhere in Newfoundland (approximately 70 localities).

#### **Collection localities**

Some records are based on photos posted online on three web sites:

- IoN Insects of Newfoundland Facebook group (https://www.facebook.com/groups/ 717236451733098/)
- (2) iNat iNaturalist (https://inaturalist.ca/observations?place\_id=67128&subview=table& taxon\_id=130414)
- (3) BG Bugguide (https://bugguide.net/node/view/1825243/bgpage)

NEWFOUNDLAND: Bay Roberts (124 Conception Bay Highway), 47.58° N, 53.28° W, 4 September 2022, sweep of trailside plants, B. Hicks [2, BHC]; Bell Island, 31 December 2020, S. Boone [1, IoN photo]; Ibid., 10 January 2021; Ibid., 10 October 2022; Carbonear (8 Bond St.), 47.74° N, 53.22° W, 12 August 2021, B. Hicks [1, BHC]; Ibid., 15 August 2021, B. Hicks [1, NLC;



**Figure 3.** Maximum likelihood tree of *Adalia* spp. using 658-bp mitochondrial DNA from the 5' barcode region of *CO*1. Bootstrap values generated by Ultrafast (Hoang *et al.* 2018) bootstrapping with 1000 replicates, with values greater than 50 reported and values less than 50 replaced by a hyphen (-). Dashed box contains all *A. decempunctata* sequences. Coloured branches represent specimens barcoded for this study (Table 1). Coloured bar indicates country of collection for all *A. decempunctata* specimens. Collapsed nodes represent polytomy due to zero sequence divergence. The tree was generated in IQ-Tree with substitution model determined by ModelFinder (Trifinopoulos *et al.* 2016; Kalyaanamoorthy *et al.* 2017): Transversion model with empirical base frequencies and gamma-distributed rates among sites across four categories (TVM + F + G4). The tree was edited with FigTree, version 1.4.4 (Rambaut 2007), and Affinity Photo (Serif 2023).

BOLD no.	Sample ID	Identity	Collection date	Locality	Repository
ADDEC001-23	4001	A. bipunctata	18 May 2020	Edmonton, AB	UASM
ADDEC002-23	70001	A. bipunctata	July 2021	St. John's, NL	NFRC
ADDEC003-23	70002	A. bipunctata	July 2021	St. John's, NL	NFRC
ADDEC004-23	70003	A. bipunctata	July 2021	St. John's, NL	NFRC
ADDEC005-23	70106	A. decempunctata	3 June 2020	St. John's, NL	NFRC
ADDEC006-23	70107	A. decempunctata	3 June 2020	St. John's, NL	NFRC
ADDEC007-23	70108	A. decempunctata	3 June 2020	St. John's, NL	NFRC

**Table 2.** Barcode of Life Datasystems (BOLD) accession numbers, sample identification numbers (sample ID), and collection data for specimens of *Adalia bipunctata* (Linnaeus) and *Adalia decempunctata* (Linnaeus) DNA barcoded for this study. AB, Alberta; NL, Newfoundland and Labrador.

UASM, University of Alberta Strickland Entomological Museum, Edmonton; NFRC, Northern Forestry Centre Insect Collection, Edmonton.

MUN Accession No. 08-4516]; Carbonear (10 Chapel Place), 47.740° N, 53.238° W, 8 April 2021 [1, BHC]; Conception Bay South, 17 July 2019 [1, iNat photo]; Ibid., 21 July 2020, K. Earle [1, ION photo]; Ibid. 13 February 2021, N. Kelley [1, IoN photo]; Conception Bay South, Manual's River, September 2002 [2, NLC; MUN Accession Nos 08-4473 and 08-4474]; Harbour Grace, 47.69° N, 53.21° W, 4 September 2022, B. Hicks [1, BHC]; Logy Bay, 2 July 2020, M. Erbland [1, IoN photo]; Ibid., 30 July 2020; Logy Bay, 31 July 2020 [1, iNat photo]; Mount Peart, 26 July 2020, A. Garrison [1, IoN photo]; Mount Pearl (16 Mundon Dr.), 7 July 2021, R. Parks [1, BHC]; Paradise, 7 February 2021, L.A. Payne [1, IoN photo]; Paradise (18 Hudsonberry Lane), 7 February 2021, B. Hicks [1, BHC]; South River (Love Lane), 47.540° N, 53.271° W, 4 September 2022, sweep of roadside plants, B. Hicks [2, BHC]; Spaniard's Bay (89 Seymour's Rd.), 47.629° N, 53.625° W, 5 September 2021, B. Hicks [1, BHC]; St. John's, 47.5658° N, 52.7253° W, 12 September 1995 [1, NFRC]; St. John's, 16 August 2019 [1, iNat photo]; Ibid., August, 2019; Ibid., 31 July 2020; Ibid., 3 August 2020; Ibid., 7 November 2020; Ibid., 5 October 2022; Ibid., 23 July 2023; Ibid., 13 August 2023; St. John's (11 Parson's Place), 20 June 2020, B. Hicks [1, BHC]; St. John's (116 Quidi Vidi Rd.), 8 August 2020, D. Foley [1, BHC]; St. John's (20 Vinnicombe St.), 3 June 2020, J. Clarke [5, BHC; 1, CNC; 1, NFRC; 1, NLC – MUN Accession No. 08-4503; 4, iNat photos; 3, BG photos]; St. John's (3 Ross Rd.), 6 June 2021, B. Hicks [1, BHC]; St. John's, Botanic Garden, 23 August-4 September 1999, D. Larson [1, NLC; MUN Accession No. 08-4506]; St. John's, Bowering Park, 17 June 2018, T.L. Rimmer [1, IoN photo]; Ibid. site 2, 47.5278° N, 52.7480° W, 16 August 2022, sweep of park vegetation, D. Langor [1, NFRC; 2 UASM]; Ibid., site 1, 46.5273° N, 52.7517° W, 2 August 2022 [5, NLC; 1, CNC]; Ibid., 19 October 2019 [1, iNat photo]; St. John's (downtown), 31 May 2021, S. Gallant [1, IoN photo]; St. John's, Kilbride (26 Lannon St.), 3 August 2022, B. Hicks [1, BHC]; St. John's, Long Pond, 14 October 1997 [1, NFRC]; Ibid., September 2000 [1, NFRC]; St. John's (MacDonald Drive), 12 June 2020, K. Oliver [2, IoN photo]; St. John's (MUN), 31 July 2020, R. Maddigan [1, IoN photo]; St. John's (Topsail Rd.), November 2001 [1, NLC; MUN Accession No. 08-4507); St. John's (Wadland Cres.), 28 July 2022, C. Baggs [1, IoN photo]; Torbay, 22 November 2020, D. Peyton [1, IoN photo]; Torbay, 23 September 2022 [1, iNat photo].

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