Appendix S1 – Identification of army ant species

Hunting habits die hard:

Conserved prey preferences in army ants across two distant neotropical rainforests

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Identification of army ant species

Recent phylogenomic and population genomic data on *Eciton* army ants have provided strong evidence that more species exist in this genus than are currently described (Winston et al., 2016). Identifying a species is thus challenging given the current taxonomic (non-updated) species status in the last revisions (Borgmeier, 1955; Watkins, 1976). We therefore provide details on species identifications below and supplemented our morphological identifications with DNA barcode analysis and biogeographic distribution data. We used the generic key of Borowiec (Borowiec, 2016), and the species keys of Longino (Longino, 2010), Watkins (Watkins, 1976; Watkins 1977; Watkins, 1993), and Borgmeier (Borgmeier, 1955). We further considered the recent biogeographic distribution maps provided by the phylogenomic study of *Eciton* army ants by Winston et al. (2016). We identified army ant workers of the following 14 army ant species from the two study sites: *Cheliomyrmex andicola* Emery, 1894, *Eciton burchellii* (Westwood, 1842) (subspecies *E. burchellii foreli* Mayr, 1886), *Eciton hamatum* (Fabricius, 1782), *Eciton lucanoides* Emery, 1894, *Eciton mexicanum* Roger, 1863, *Eciton vagans* (Olivier, 1792), *Labidus praedator* (Smith, F., 1858), *Labidus spininodis* (Emery, 1890), *Neivamyrmex asper* Borgmeier, 1955, *Neivamyrmex curvinotus* Watkins, 1994, *Neivamyrmex gibbatus* Borgmeier, 1953, *Neivamyrmex pilosus* (Smith, F., 1858), *Nomamyrmex esenbeckii* (Westwood, 1842), and *Nomamyrmex hartigii* (Westwood, 1842).

To facilitate future taxonomic work, we have uploaded specimen information for army ant workers collected in the present study to BOLD Systems (248 specimens from Ecuador; 356 specimens from Costa Rica). The respective voucher specimens have been deposited at the TU Darmstadt Insect Collection. We plan to distribute these samples in the future and deposit vouchers at various natural history museums. Any updates regarding changes in depository information will be reflected in BOLD Systems. GenBank accession numbers are provided on the BOLD Systems website. Voucher images for 148 specimens (N = 707 images representing 18 army ant species) have been uploaded, encompassing 2-D overview images as well as close-up images of morphological characters used for identifications. Additionally, we have deposited *COI* barcodes of 155 army ant workers into BOLD Systems under the data set 'DS-NEOARMY.' Protocols for acquiring *COI* barcodes are detailed in the main article.

To identify army ants genetically, we ran a BOLD Taxon ID tree of doryline army ants, based on the neighbour-joining algorithm and Kimura 2 parameter as distance model, including a total of 2,277 public *COI* barcodes (including the ones acquired in this work; see tree below). DNA barcodes confirmed our morphological identifications in that the species names of barcoded specimens acquired in the present work clustered closely together with records of the respective species in the database. A few records clustered into distinct genetic groups (or BINs) and we re-inspected these workers more closely to screen for consistent morphological differences. In the following we present some notes on the identification of army ant taxa based on DNA barcodes, morphological identifications, and the distribution of each species. Some uncertainties remain in the assignment of species and more detailed taxonomic studies on army ant species and their distributions are clearly necessary.

***Eciton burchellii foreli* Mayr, 1886:** *Eciton burchellii* comprises two subspecies found in Costa Rica (Longino, 2010), most likely representing distinct species (Winston, 2016). They can be easily distinguished by the colour of the gaster (Borgmeier, 1955; Longino, 2010). The maroon hue of the gaster in workers (excluding majors and submajors) observed at La Selva in Costa Rica led to the identification of *Eciton burchellii foreli*. This also agrees with the known distribution of the subspecies. Regarding army ants, Costa Rica, together with Panama, is divided into two main biogeographic regions: the part west of the volcanic front, known as the 'Chrotega block', and the part east of it. The western region is primarily inhabited by the army ant subspecies *Ec. burchellii parvispinum* Forel, 1899, whereas the eastern region is predominantly inhabited by the subspecies *Eciton burchellii foreli* Mayr, 1886

(Watkins, 1976; see also www.antmaps.org). While the distribution of *Ec. burchellii parvispinum* stretches further north up to Mexico (Watkins, 1976), the distribution range of *Ec. burchellii foreli* mostly goes further south, with records in Panama, Colombia, Venezuela, and Ecuador (Watkins, 1976). The Andes seem to be a barrier for the (sub)species, so that it occurs further south only on the western side of the Andes (Watkins, 1976). According to Winston et al. (2016), *Eciton burchellii foreli* is distributed from Nicaragua in Central America to Colombia and Venezuela in South America. Although Winston et al. (2016) did not include records from Western Ecuador (west of the Andes), we consider it highly likely that the distribution of *E. burchellii foreli* extends from the Chocó–Darién moist forests in Colombia into Ecuador, where the Reserva Río Canandé is located.

Our morphological and DNA barcode analysis further supported the identification as *Ec. burchellii foreli*. Specimens from the Reserva Río Canandé in Ecuador exhibited a similar maroon gaster, and further, we identified a close DNA barcode similarity (98.45% average sequence similarity) to specimens collected at La Selva, providing evidence that we indeed compared the same (sub)species in this study (see tree below), as expected by the biogeographic considerations described above. In contrast, specimens of the subspecies *Eciton burchellii parvispinum* collected in Monteverde, Costa Rica, displayed a much lower DNA barcode similarity to both Costa Rican and Ecuadorian specimens (see tree below). Noteworthy, genetic evidence suggests the presence of a third species in Amazonia (maybe the subspecies *Ec. burchellii* s. str.), on the other side of the Andes (Winston et al., 2016).

Eciton mexicanum* Roger, 1863*: The identification of subspecies is more challenging in *E. mexicanum* than in *E. burchellii* (see Longino, 2010), in particular as queens, males and also majors were not available (Borgmeier, 1955). Similar to *Eciton burchellii*, two closely related sister species of *Eciton mexicanum* — probably the currently described subspecies *E. mexicanum* s. str. and *E. mexicanum panamense* (Watkins, 1976) — appear to coexist in Central America (Winston et al., 2016). However, only one of these species seems to extend into South America. We are uncertain about which subspecies we studied at La Selva and at the Reserva Río Canandé. While the biogeographic distribution pattern suggests that we studied the same species in both locations (see discussion on geographic barriers above), DNA barcode analyses were unclear. The *COI* barcodes of the only sequenced *E. mexicanum* worker (sample ID: EV1000_wo010) collected at the Reserva Río Canandé matched more closely with specimens from Monteverde in Costa Rica (sample codes cvb800) and Nicaragua than with those collected at La Selva (see tree below), but still showed a 7% sequence divergence to the Monteverde specimens. However, several genetic clusters exist in this species, and only more samples from a broader geographic range, coupled with detailed morphological studies, might allow us to differentiate distinct species in this group. For the present work, we consider the samples from La Selva and the Reserva Río Canandé as a single species. As we have deposited voucher material and DNA barcodes, future work might allow us to verify this assumption.

***Eciton vagans* (Olivier, 1792):** *Eciton vagans* possesses a very similar biogeographic distribution pattern to *E. mexicanum*, with two species (probably the subspecies *Ec. vagans angustatum* and *Ec. vagans mutatum*; Watkins, 1976) being found in Central America. The distribution of only one of them extends into South America (Winston et al., 2016). The distinction of these two potential subspecies is challenging or even impossible without queens and males (Longino, 2010). We agree with Longino (2010) that the lengths of the anteroventral postpetiolar tooth of workers does not seem to be a reliable diagnostic character to distinguish the subspecies (Longino, 2010). Borgmeier (1955) suggested that workers of the different subspecies could be distinguished using this character, although he also noted that subspecies characters are not well developed and difficult to detect. We found considerable variation in the teeth of workers, from a pronounced teeth to a nearly absent teeth (20 inspected workers from Ecuador; 12 inspected workers from Costa Rica). Several *COI* barcode clusters existed in this species (see tree below). DNA barcodes matched less well between the specimens collected at La

Selva and those found at the Reserva Río Canandé than in *Ec. burchellii*, but still showed an average sequence similarity of 95.89%. The biogeographic distribution patterns and DNA barcode similarity suggested to us that we studied the same species in both locations.

***Eciton dulcium* Forel, 1912:** While previous morphological work indicated the existence of two *Ec. dulcium* subspecies (*Ec. dulcium* s. str. and *Ec. dulcium crassinode*; Borgmeier, 1955; Watkins, 1976), the phylogenomic data of Winston et al. (2016) detected only a single *E. dulcium* species with a wide distribution from Central America to South America. Without queens and males present it is infeasible to us to distinguish possible subspecies with the existing key (Borgmeier, 1955). The subspecies *Ec. dulcium crassinode* was described from Panama and Costa Rica, and we consider it most likely that this subspecies was studied at La Selva.

***Eciton lucanoides* Weber, 1949:** While the sample size of sequenced *Ec. lucanoides* in Winston et al. (2016) was relatively low, they were able to distinguish two distinct species, one in Central America and one in Southern Amazonia. As discussed above for the distribution of *Ec. burchellii foreli*, we consider it likely that the Central American *Ec. lucanoides* species extends into South America via the Chocó–Darién moist forests into Colombia and Ecuador. Support for this comes from the DNA barcode data, as two specimens collected at the Reserva Río Canandé showed close DNA barcode matches to specimens collected at La Selva (97.16% average sequence similarity; see tree below). Two subspecies are distinguished: *E. lucanoides* s. str. and *E. lucanoides conquistador* (Borgmeier, 1955). Separating these subspecies was impossible to us without having a reference collection, and we thus refrain from identifying the herein studied subspecies. A discussion on the subspecies status and the species status of *Ec. jansoni*, a species described based on a male that is likely the same species as *Ec. lucanoides*, was provided by Longino (2010). Longino concludes that more work is clearly needed on the character variation of this species across its biogeographic range. Due to the biogeographic considerations provided above and the DNA barcode similarities, we consider it likely that we studied the same species at both study sites.

***Eciton hamatum* (Fabricius, 1782):** Two genetic lineages of *E. hamatum* were detected by Winston et al. (2016), and these had a similar distribution to the ones described above for *E. lucanoides*. Two records existed from Northern Colombia (Winston et al., 2016), and based on our biogeographic considerations, we expect this species to extend to the Chocó moist forests in Ecuador. This is again supported by a high DNA barcode similarity between the Costa Rican and the Ecuadorian samples (98.23% average sequence similarity; see tree below). We are thus confident that the herein compared *E. hamatum* specimens do indeed belong to the same species.

No subspecies are described for *Ec. hamatum*. However, the army ant *Ec. drepanophorum* is morphologically very close to and difficult to distinguish from *Ec. hamatum*. Since *Ec. drepanophorum* is anticipated to be mostly distributed in the Amazonian region according to Borgmeier (1955), Watkins (1976), and Winston et al. (2016) (see also antmaps.org), we infer that the specimens we gathered in this study, which took place in Central America and western Ecuador, belong to the species *E. hamatum*. However, using the species keys of Watkins (1976) and Borgmeier (1955), we would have probably identified some of the individuals as *Ec. drepanophorum*, because in some workers the second segment of the flagellum was not longer than the apical width of the scapus and the propodeal keel was about parallel in dorsal view. However, we found intracolonial variation in these characters, with some specimens having a slender second flagellum exceeding the scape width and others having a shorter second flagellum not exceeding the scape width. This suggests to us that these characters might not represent reliable diagnostic characters. Due to the known geographic distribution pattern and the described DNA barcode matches, we are rather confident that the collected material at both study sites belongs to *Ec. hamatum*.

***Neivamyrmex asper* Borgmeier, 1955**: We acquired DNA barcodes only for specimens collected at La Selva. Additional barcodes were available only from Mexico and Nicaragua. Consequently, we identified specimens collected in Ecuador solely based on morphological characters, utilizing the keys provided by Longino (2010) and Watkins (1976). *Neivamyrmex asper* has been exclusively collected in South American and Central American locations yet (Watkins, 1976; also refer to www.antmaps.org), raising initial doubts about the robustness of the identification. However, employing the species key by Longino (2010) and Watkins (1976) suggested an identification as *Neivamyrmex asper* but uncertainty remains. This finding thus might represent a new distribution record for the species (see also https://blog.myrmecologicalnews.org/2019/06/19/a-photographic-journey-to-the-ants-of-the-ecuadorian-choco/). We have deposited DNA extracts and voucher specimens of the species, enabling future researchers to verify our species designation.

***Neivamyrmex gibbatus* Borgmeier, 1953**: When considering data from various geographic locations (Costa Rica, Panama, Venezuela, Nicaragua, Ecuador), DNA barcode analysis showed that the army ant species identified as *Neiv. gibbatus* formed several distinct genetic clusters (see tree below). We inspected 20 specimens from 11 distinct raids from Ecuador and 38 specimens from 16 distinct raids from Costa Rica. All specimens were re-identified as *Neiv. gibbatus* using the keys of Watkins and Borgmeier (Borgmeier, 1955; Watkins, 1976). The studied material was distinct from other *Neivamyrmex* species in the following combination of characters: presence of a humped mesonotum, apex of antennal scape exceeding upper margin of head, dorsal surface of propodeum about the same length as descending surface (compare with *Neiv. pseudops* on AntWeb), head and alitrunk reddish-brown (not black), and a postpetiole that is about as long as high. However, we certainly cannot rule out that several distinct species are hidden under this species name, and future work might clarify this. Genetic data of the Ecuadorian samples as well as those from Costa Rica clustered together in the Taxon ID tree but in two distinct clusters (see tree below). Whether the studied material belong to the same species thus remains uncertain. We did not detect morphological characters distinguishing these two clusters, and therefore we treat them as a single species in the present work.

Neivamyrmex curvinotus: Previous to the present work, there were no records of this species in the BOLD Systems database. We consider the army ant specimens of five different raids to belong to the species *Neivamyrmex curvinotus* (Watkins, 1993; for raid IDs see Table S1 on Dryad). This species belongs to Borgmeier's species group III, and can be distinguished from other members of this group by having a lighter coloration (yellowish to light reddish brown; e.g. *Neiv. pilosus* is much darker), a characteristic double-arched mesosoma, and a more arched propodeum. Overall, the specimens fit well to Watkin's species description and the collections site fits to the origin of type material from Peru. We thank Leonardo Tozetto for help in identifying this species. Four DNA barcodes from two distinct colonies had identical sequences and grouped into a distinct cluster in the genetic tree (see tree below).

***Neivamyrmex* MAS03**: We collected this species in Costa Rica and initially identified it as *Neiv. iridescens* using the key of Longino (2010). Although DNA barcode analysis clustered our specimens closely to this species, the barcodes were distinct and matched best to specimens denoted as *Neivamyrmex* MAS03 in the reference database, and we therefore adopted this name. Notably, specimens of this species were neither part of the Hoenle et al. (2019) study, nor were they found in the present work about Ecuadorian army ant predation. We still added the information here to facilitate future research on *Neivamyrmex* army ants. Voucher specimens and DNA barcodes were deposited.

***Neivamyrmex pilosus* (Smith, 1858):** We only found this species at La Selva. It has a wide distribution from the Southern USA to Northern Argentina (Borgmeier, 1955; Watkins, 1976). Borgmeier (1955) distinguished four subspecies: *Neiv. pilosus* s. str., *Neiv. pilosus beebei*, *Neiv. pilosus mexicanus*, and *Neiv. pilosus mandibularis*. These subspecies were distinguished based on male characters, which does not allow us to identify the subspecies studied at La Selva. Given the distribution of the other

subspecies (Borgmeier, 1955), we consider it likely that we here studied the subspecies *Neiv. pilosus mexicanus*, as this subspecies was collected at several sites in Costa Rica (Borgmeier, 1955; Longino, 2010). However, uncertainty remains, and voucher species might help disentangling the variation in this species more reliably. No barcode was acquired for this species.

***Labidus spininodis* (Emery, 1890):** We found this species in Ecuador but missed finding workers in Costa Rica, where it is also present (Longino, 2010). It has a broad distribution from Central America to South America, including records in Western Ecuador (www.antweb.org). No subspecies are described. The species is morphologically very similar to *Lab. praedator* but can easily be distinguished from the latter species by possessing a pronounced ventral petiolar spine (for differences to *Lab. coecus* see Longino, 2010). We obtained a single DNA barcode for the species from a male collected at La Selva, which most closely matched records of this species from Panama. However, DNA barcode records in *Labidus* are chaotic, with several *Lab. spininodis* clusters existing throughout the presented tree (see tree below). Similar to *Eciton*, the genus *Labidus* is in need of a taxonomic revision. We designate the species studied herein as *Lab. spininodes* due to morphological characteristics that clearly identify it as this species, but uncertainty remains whether distinct species exist under this name. Voucher specimens are available for future work, including the male specimen.

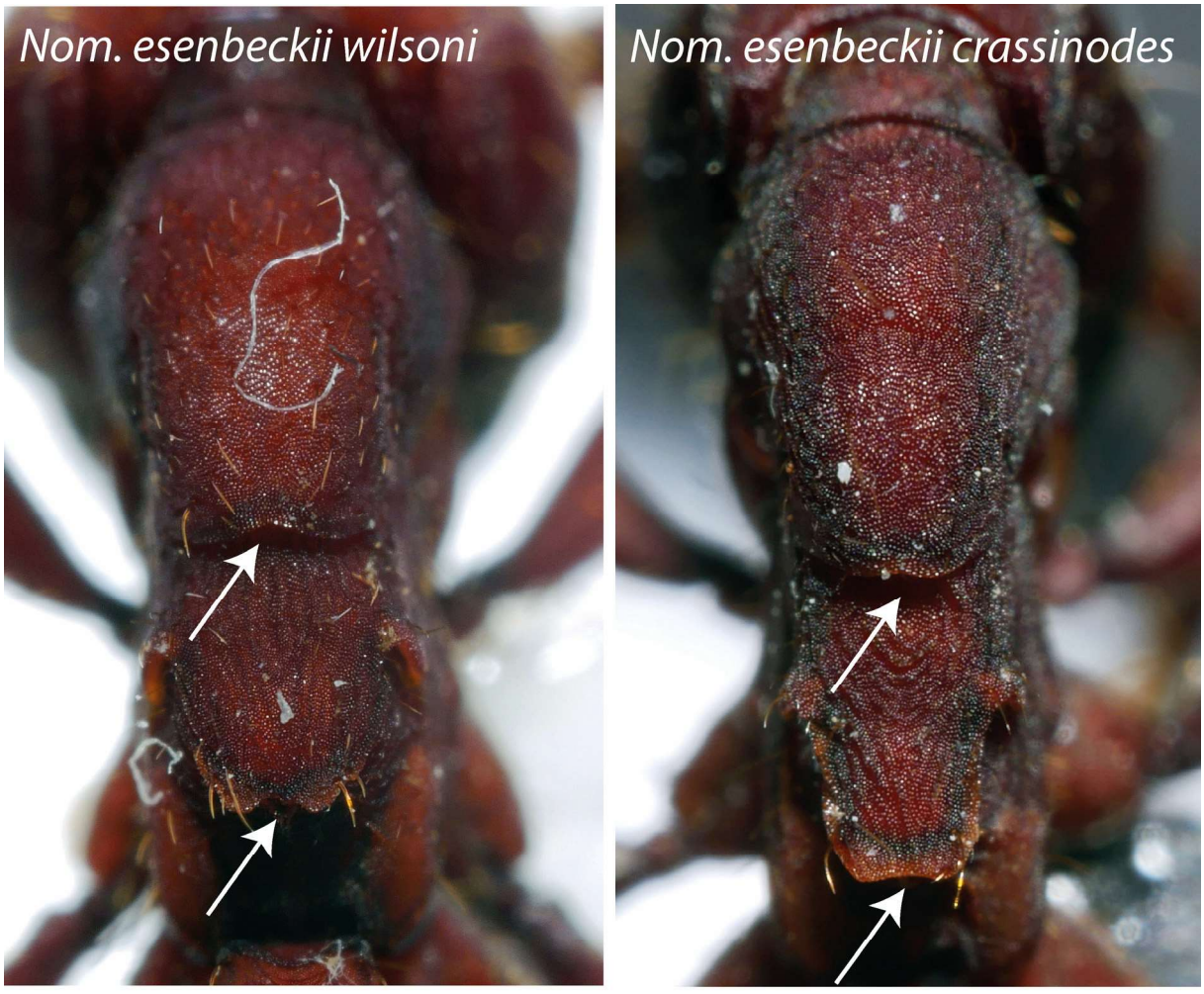
***Labidus praedator* (Smith, F., 1858):** Similar to *Lab. spininodis*, we located this species in Ecuador but regrettably did not encounter it in Costa Rica, where it is also documented (Longino, 2010). It stands out as one of the most abundant and widespread neotropical army ants, with a broad distribution from Mexico to Argentina (Longino, 2010). Although morphologically closely related to *Lab. spininodis*, workers of this species lack the ventral petiolar spine. Borgmeier (1955) described two subspecies: *Lab. praedator* s. str. and *Lab. praedator sedulous*, both with a broad distribution. We were unable to assign the collected material to a subspecies without a reference collection. Longino (2010) noted that the variation of the species needs to be more thoroughly investigated. Unfortunately, no DNA barcodes were acquired for this species, so it remains uncertain whether we have studied the same (sub)species at the two study sites, although we consider this likely due to the biogeographic distribution pattern of army ants discussed above.

***Nomamyrmex hartigii* (Westwood, 1842):** We rarely encountered this species at both two study sites. Watkins (1977) presented the distribution of the species, which ranges from Mexico to Paraguay and Southern Brazil. It can easily be distinguished from *Nomamyrmex esenbeckii* by having a transverse line without a deep groove at the back of head and by having a smooth surface on top of the petiole in contrast to *No. esenbeckii*, which has a series of ridges (rugae) at this place (Watkins, 1977; Longino, 2010). We did not acquire a *COI* barcode for this species. However, no subspecies are described, and we thus consider the species studied at La Selva and those studied at the Reserva Río Canandé as the same species for the present work.

***Nomamyrmex esenbeckii* (Westwood, 1842):** Based on the characters described above, we were able to reliably distinguish this species from *No. hartigii* at both study sites. Four subspecies are described in this species (Watkins; 1977). In our study, it appears as we have studied two distinct subspecies: *No. esenbeckii wilsoni* at La Selva Biological Station and *No. esenbeckii crassicornis* at the Reserva Río Canandé in Ecuador. The hind margin of the mesonotum in specimens from La Selva was emarginated in most major specimens (but not all), while it was rather straight in specimens from the Reserva Río Canandé in Ecuador (Watkins, 1977; Longino, 2010). An emargination was more pronounced at the hind margin of the pronotum of *No. esenbeckii wilsoni*, which seemed to be a consistent trait among major workers between the two populations (Figure S1). Mostly, *No. esenbeckii crassicornis* exhibited a more pronounced vertex flange than the specimens identified as *No. esenbeckii wilsoni*, but this character was more variable than the shape of the mesonotum. We observed a relatively strong difference in *COI* barcodes between a specimen collected at the Reserva Río Canandé in Ecuador and

other DNA barcodes identified as *No. esenbeckii wilsoni* from Panama (see tree below). Hence, doubts remain about the species status, but for the present work, we denote specimens collected at the two study sites as *Nom. esenbeckii*. Voucher specimens have been deposited and might allow to clarify the species status in the future. Below are images of two major workers illustrating the differences between subspecies.

Figure S1. Comparison of the hind margins of the mesonotum and propodeum of two *Nomamyrmex esenbeckii* major workers. Left: *Nom. esenbeckii wilsoni* collected at La Selva Biological Station. Right: *Nom. esenbeckii crassinodes* collected at Canandé. Specimen IDs are given below images. Images: C. von Beeren



sample ID: NO11wo011; La Selva Biol. St., Costa Rica

sample ID: NO100_Ecprey145; Res. Río Canandé, Ecuador

Supplemental references

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BOLD TaxonID Tree

Records uploaded in the present work are highlighted in green color. For each record the species name,the BOLD process ID, the sample IDs, the country of origin, the exact collection site, and the BIN ID (if available) are given, separated by vertical lines. Specifications on the tree analysis are given below.

Title : Tree Result - Search: Process IDs; Include public records (1131 records returned) (1131 records selected)

Date : 10-Jul-2023

Data Type : Nucleotide

Distance Model : Kimura 2 Parameter

Marker : COI-5P

Colourization : Barcode Cluster (BIN)

Label : Sample ID

Label : Process ID

Label : Taxon

Label : Country

Label : Exact Site

Label : Barcode Cluster (BIN)

Sequence Count : 1099

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Unidentified : 123

BIN Count : 211

Search: Mon Jul 10 09:40:59 2023

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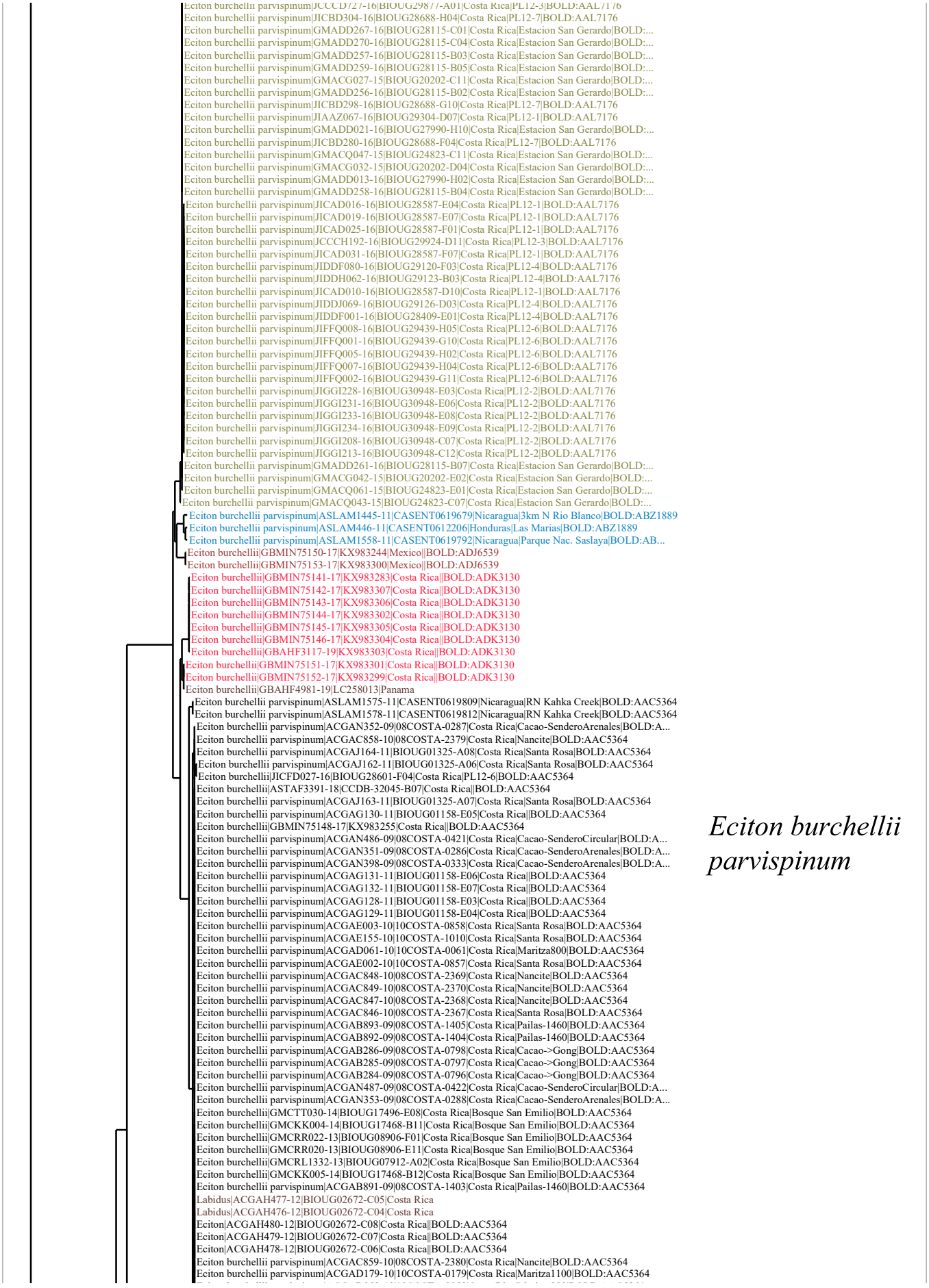

Eciton burchellii parvispinum

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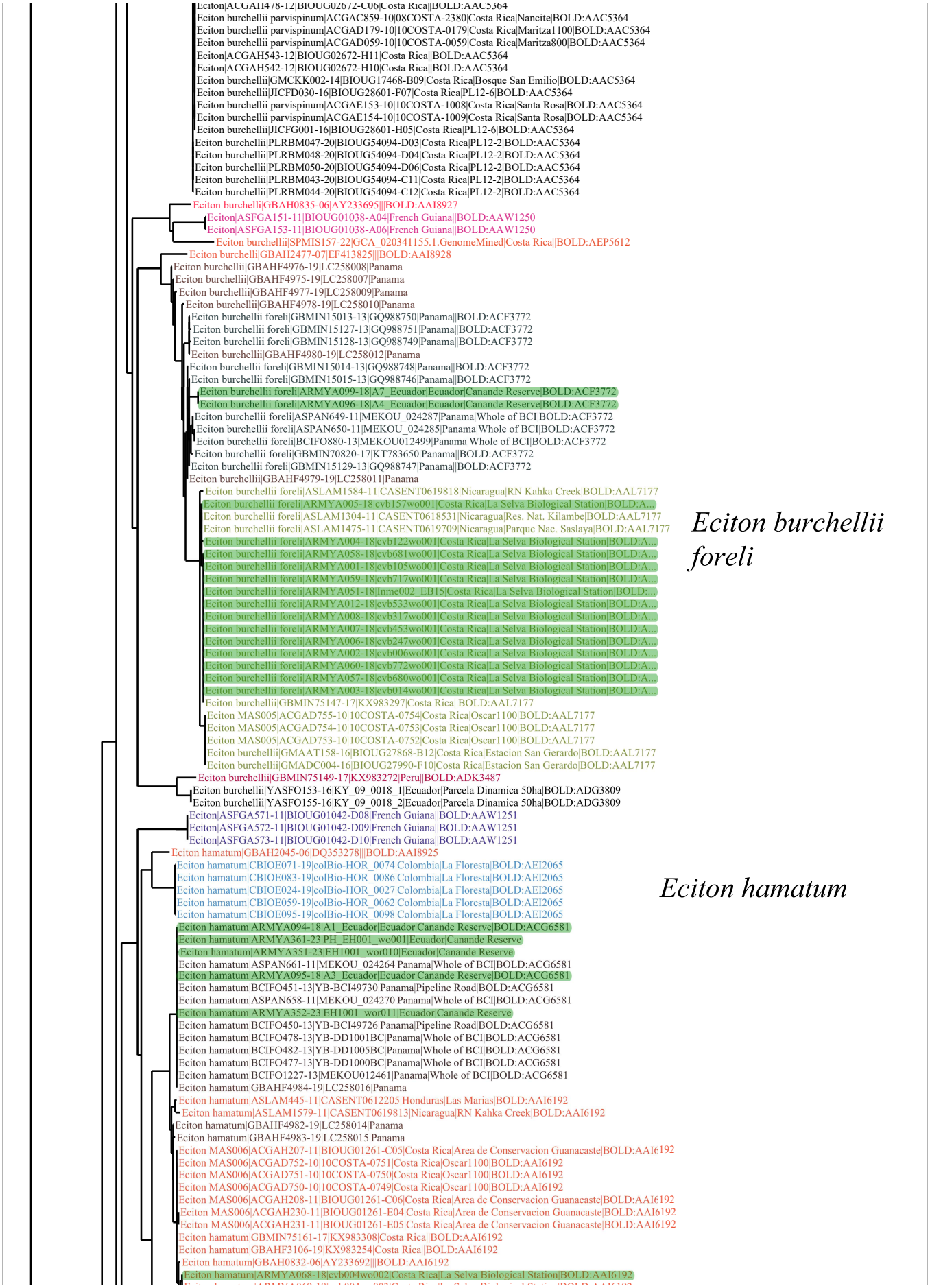

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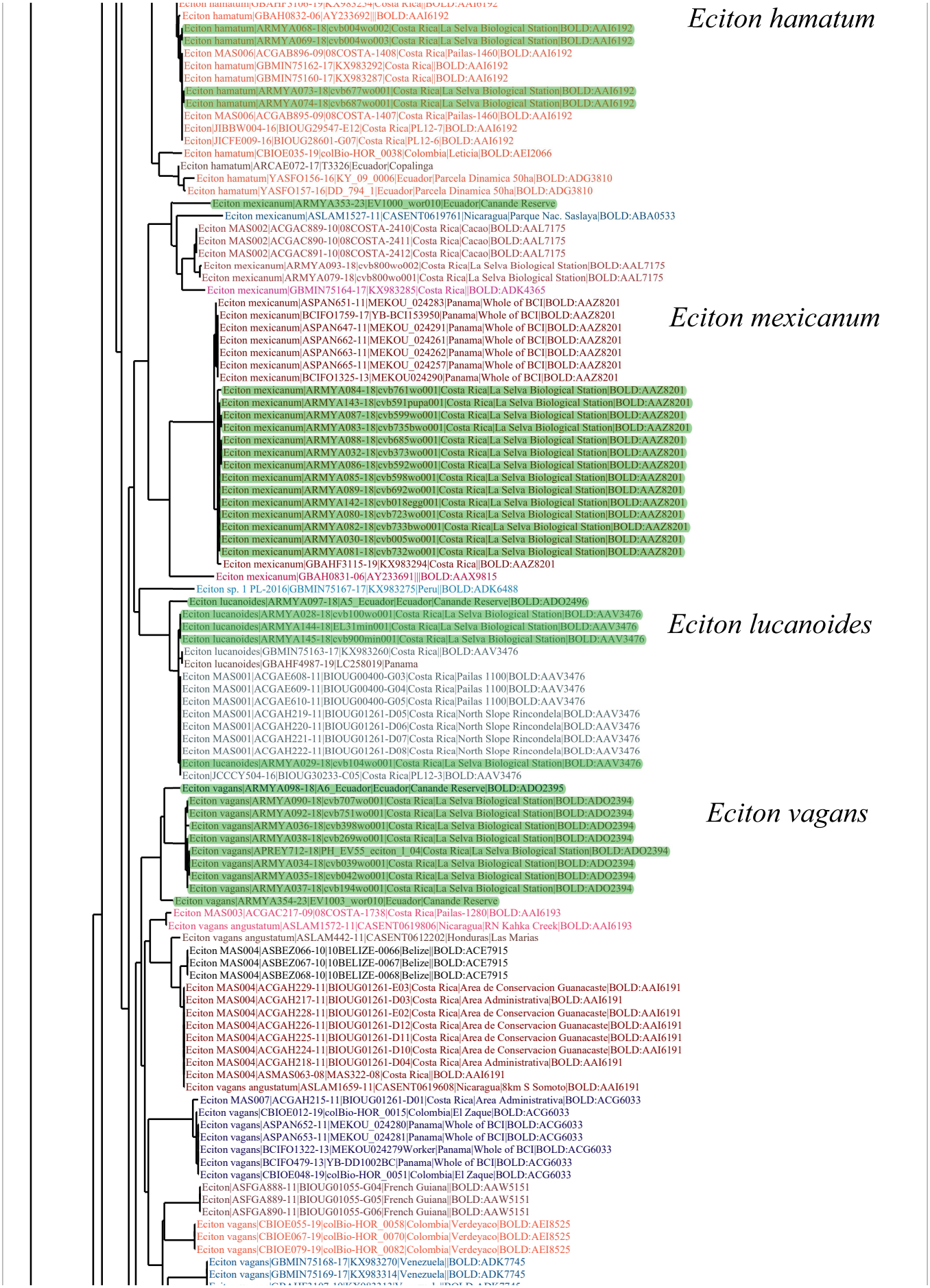

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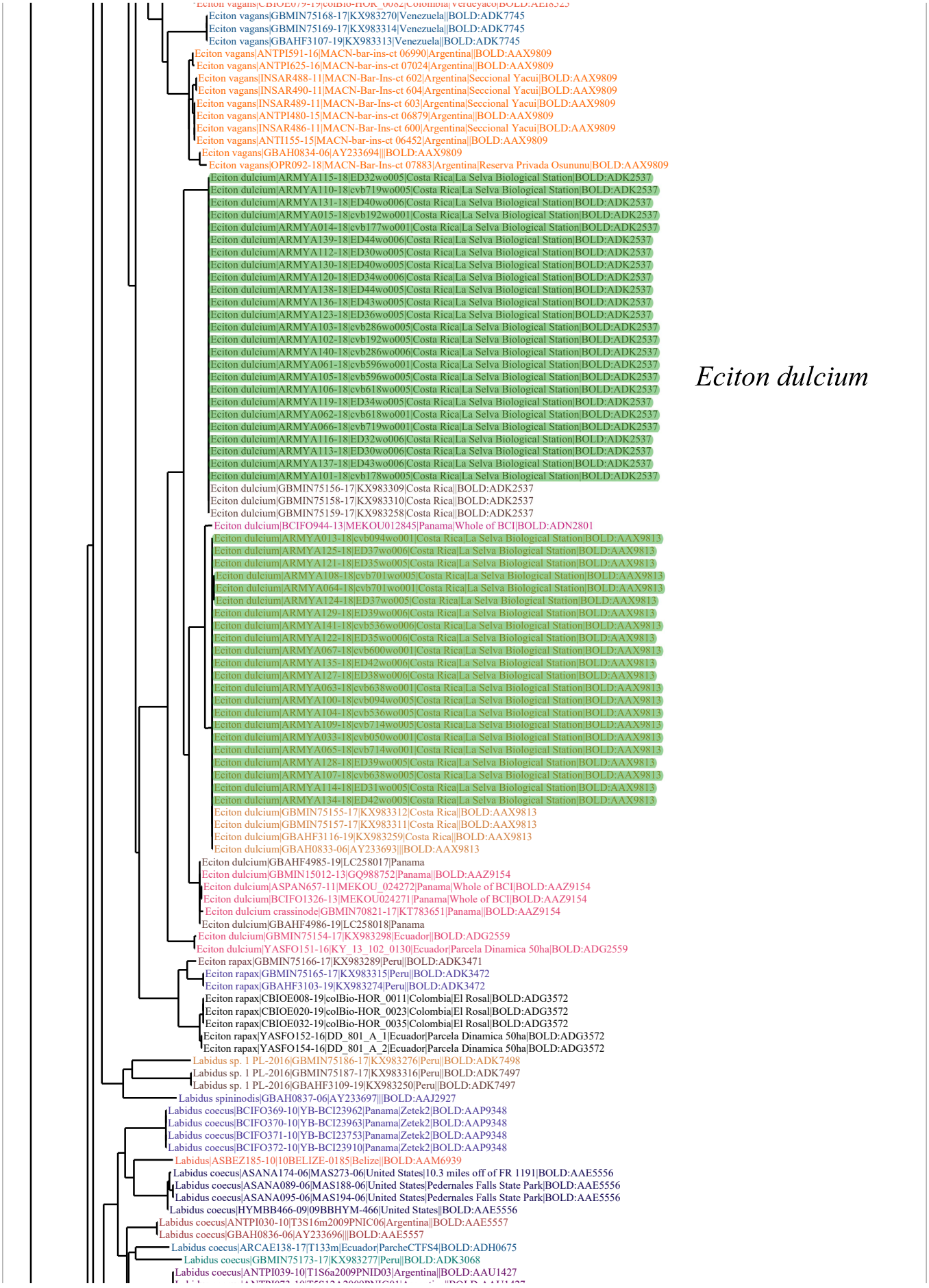

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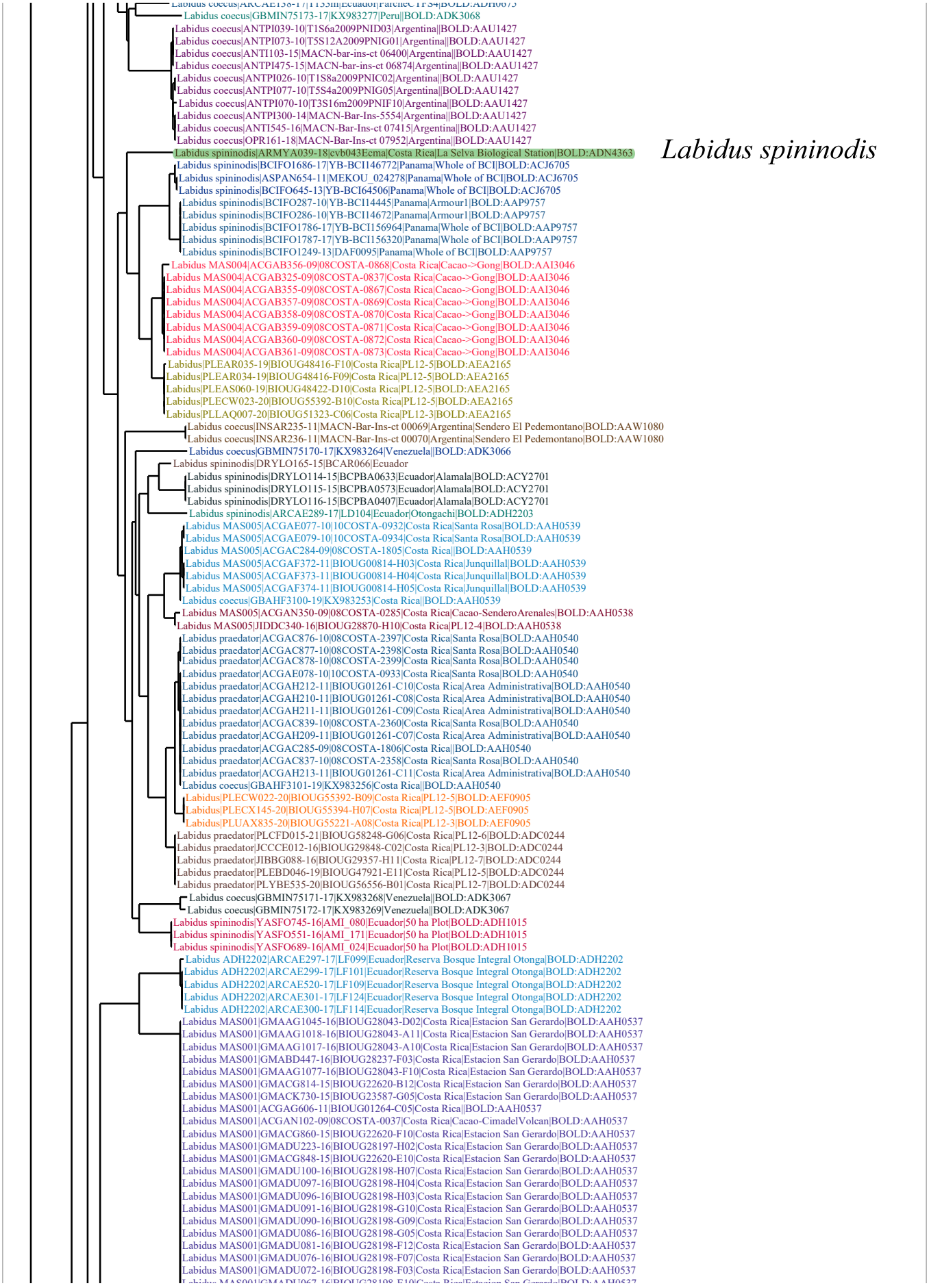

Labidus spininodis

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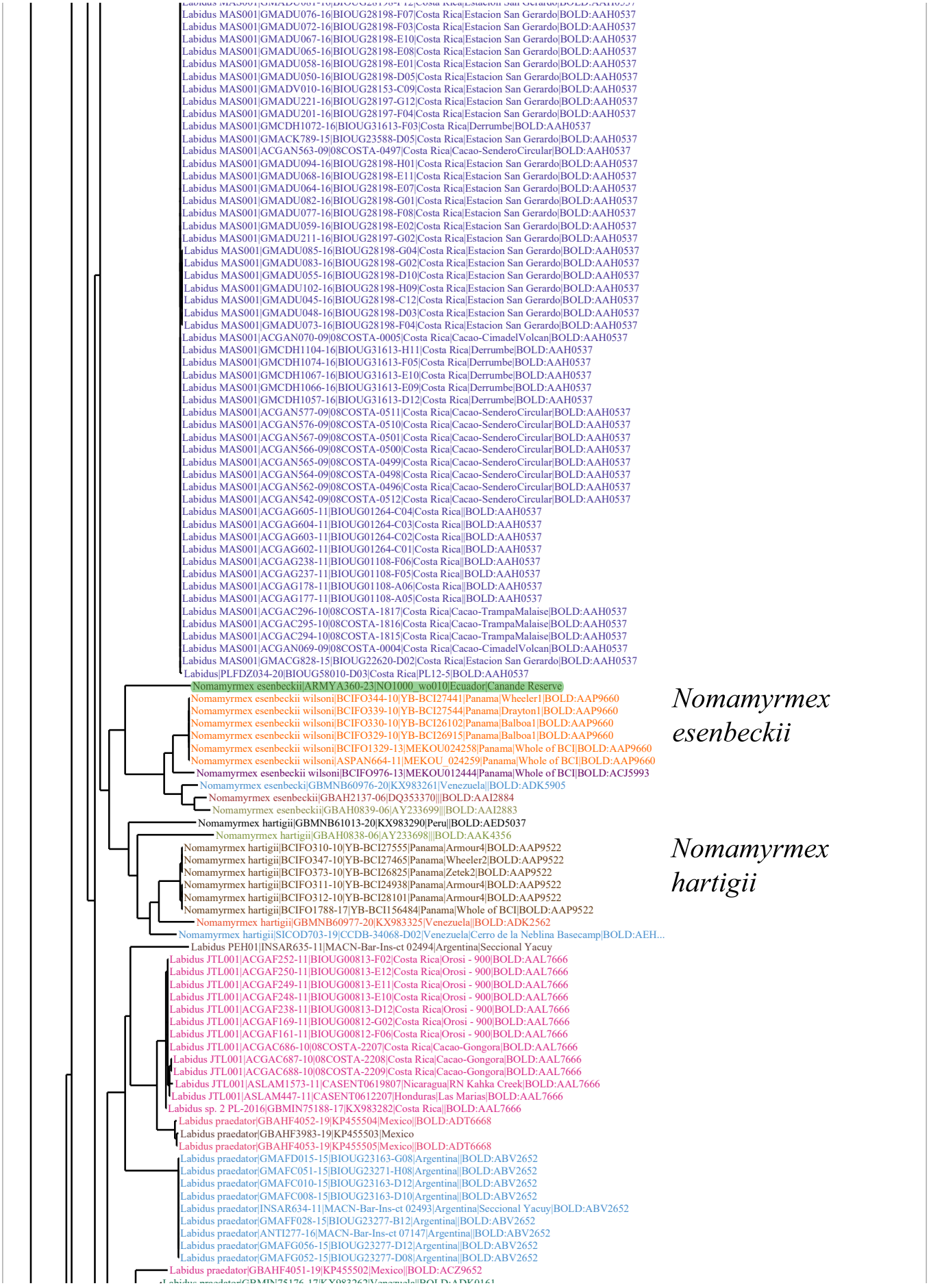

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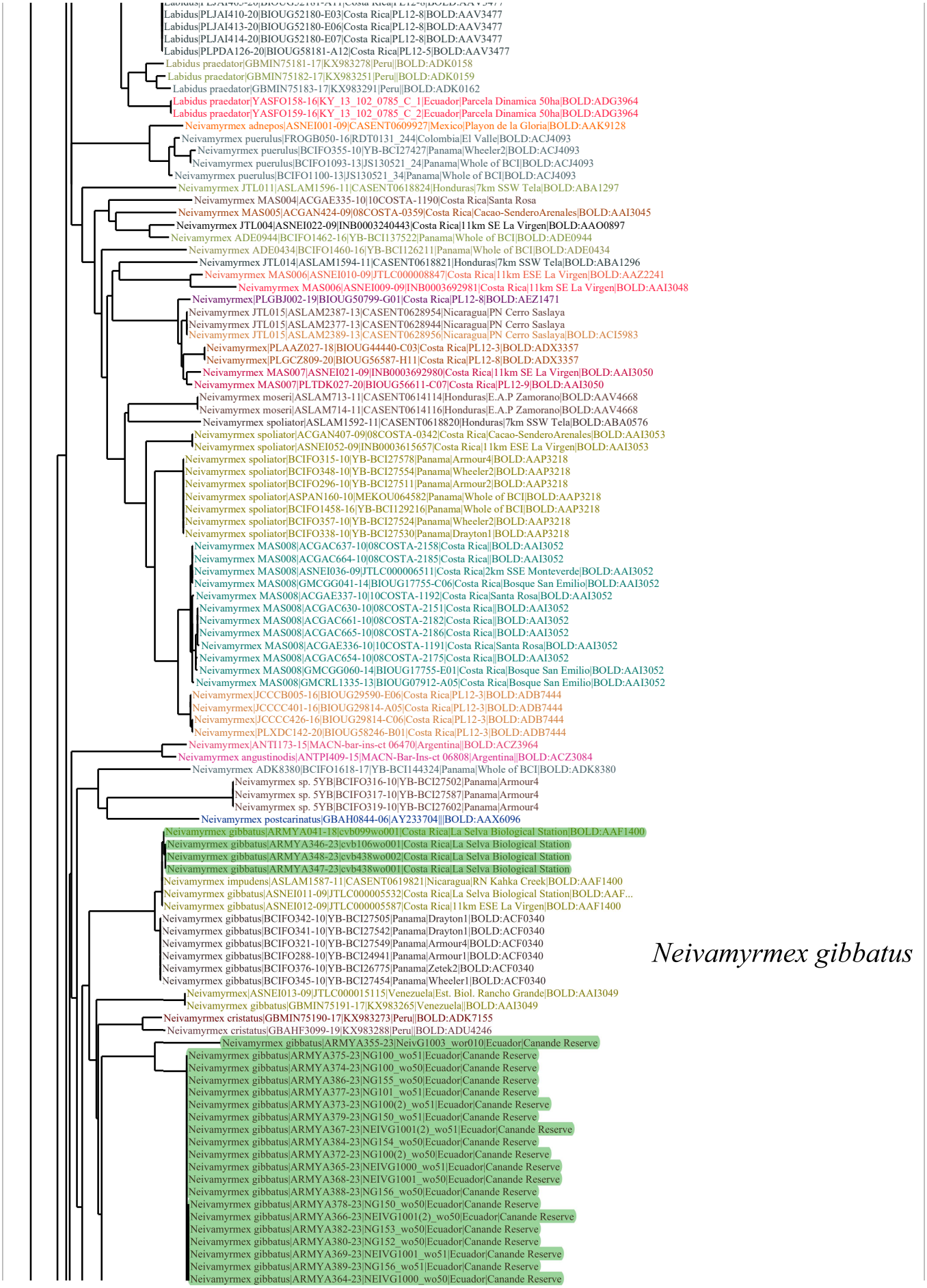

Neivamyrmex gibbatus

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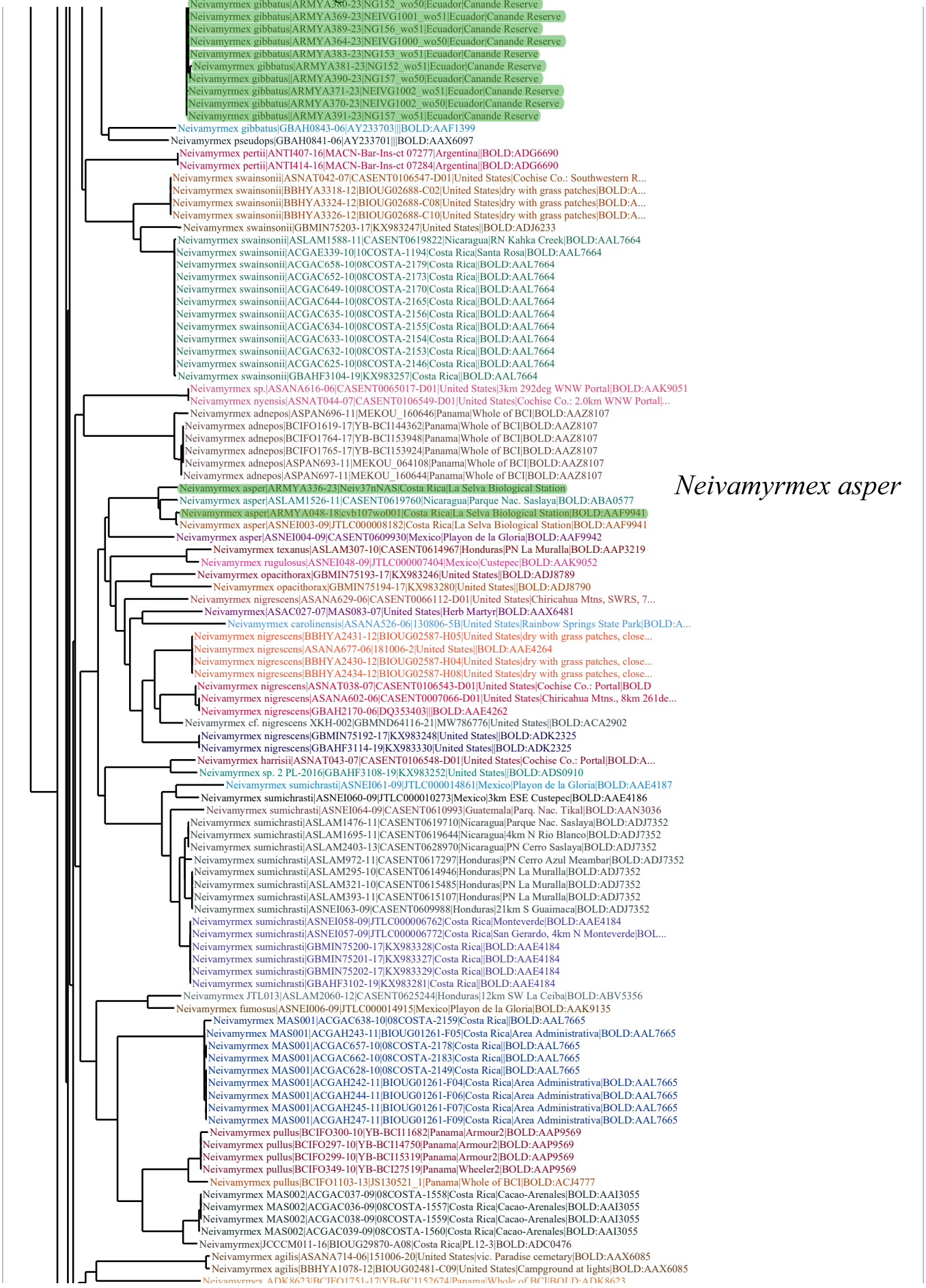

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