## Distributional Boundaries of the Geographic Races of Damaster blaptoides (Coleoptera, Carabidae) in Northeastern Japan as Deduced from Mitochondrial ND5 Gene Sequences

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Abstract The phylogenetic relationships of the geographic races of *Damaster blaptoides* inhabiting Hokkaido and the northeastern Tôhoku district, respectively, are analysed using mitochondrial NADH dehydrogenase subunit 5 (ND5) gene sequences. The distributional boundaries of the races do not coincide with those settled for the subspecies. The phylogenetic trees suggest that *D. b. viridipennis* inhabiting north of the Yoneshirogawa–Mabechigawa (Y–M line) is consubspecific with *D. b. rugipennis* in Hokkaido, while that inhabiting south of the line is distinct either from the above race, or from *D. b. babaianus* distributed in the southern Tôhoku district. The trees also suggest that *D. b. rugipennis* in Hokkaido would have been established by invasion of *D. b. viridipennis* of the northern Tôhoku population through the past land-bridges between the Tsugaru Peninsula and Hokkaido.

#### Introduction

Damaster (s. str.) has been treated as a single species, blaptoides, consisting of seven to eight subspecies or geographic races (NAKANE, 1960; ISHIKAWA, 1991). According to these morphological studies, D. b. rugipennis and D. b. viridipennis are distributed in Hokkaido and in the northern Tôhoku district, respectively, separated by the Tsugaru Straits. The molecular phylogenetic analysis has shown that the distribution of Damaster blaptoides is tightly linked to the geohistory of the Japanese Islands. The Damaster ancestor first split into two lineages, eastern (E) and western (W), which were further divided into three [HKD (Hokkaido), NTK (northern Tôhoku) and STK (southern Tôhoku)] and five (KTO, CBU, KII, WJN and KYU) geographically linked sublineages (or races), respectively (SU et al., 1998). SU et al. (1998) also found that the so-called D. b. viridipennis specimens from the northernmost localities in the Tôhoku district form a cluster HKD with D. b. rugipennis in Hokkaido, separated from

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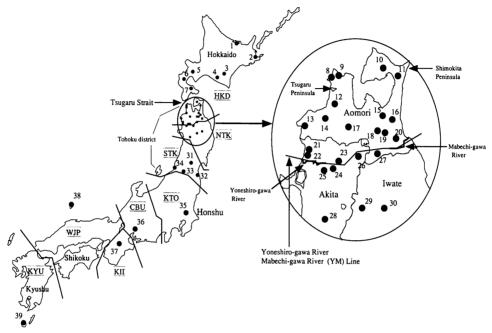


Fig. 1. Locality map of *Damaster* specimens used in this study. Locality numbers correspond to those shown in Table 1 and in Fig. 2. Three-letter codes showing the geographic races (settled by the molecular phylogeny) and the distributional boundaries of the races are according to SU *et al.* (1998).

what has been identified with *D. b. viridipennis* in NTK. Because of the shortage of specimens from the northern Tôhoku region, the distributional boundary between HKD and NTK has remained somewhat ambiguous. In this study, we have analysed the ND5 gene sequences of additional 15 specimens from this region and established a nearly complete distributional boundary between the two races.

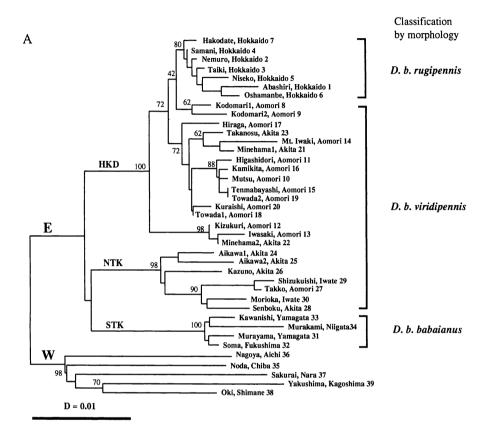
#### **Materials and Methods**

Fifteen specimens of *Damaster blaptoides* were collected from various parts of the northern Tôhoku district (Table 1 and Fig. 1), and were used for analyzing the ND5 gene sequences. A single individual from each locality was used for extraction of the total DNA from thorax muscle by proteinase K digestion (KIM *et al.*, 1999). For PCR amplification, sequencing and phylogenetic analyses, see SU *et al.* (1996 a, 1998). Phylogenetic trees were constructed by unweighted pair-group method with arithmetic mean (UPGMA) (KUMAR *et al.*, 1993), and neighbor-joining (NJ) method (SAITOU & NEI, 1987).

Table 1. List of specimens analysed.

Scientific name by morphology	Locality number	Locality	DDBJ/EMBL/GenBank Accession No.
D. b. rugipennis	1*	Abashiri-Shi, Hokkaido	AB013858
	2*	Nemuro-Shi, Hokkaido	AB013859
	3*	Taiki-Cho, Hokkaido	D50351
	4*	Samani-Cho, Hokkaido	AB013860
	5*	Niseko-Cho, Hokkaido	AB013861
	6*	Oshamanbe-Cho, Hokkaido	AB013862
	7*	Hakodate-Shi, Hokkaido	AB013863
D. b. viridipennis	8	Kodomari-Mura 1, Aomori Pref.	AB031304
	9	Kodomari-Mura 2, Aomori Pref.	AB031305
	10	Mutsu-Shi, Aomori Pref.	AB031306
	11*	Higashidori-Mura, Aomori Pref.	AB013864
	12*	Kizukuri-Cho, Aomori Pref.	AB013865
	13*	Iwasaki-Mura, Aomori Pref.	AB013866
	14*	Iwaki-Cho, Aomori Pref.	D50428
	15	Tenmabayashi-Mura, Aomori Pref.	AB031307
	16	Kamikita-Cho, Aomori Pref.	AB031308
	17	Hiraga-Cho, Aomori Pref.	AB031309
	18	Towada-Shi 1, Aomori Pref.	AB031310
	19	Towada-Shi 2, Aomori Pref.	AB031311
	20	Kuraishi-Mura, Aomori Pref.	AB031312
	21	Mizusawa-Gawa 1, Minehama-Mura, Akita Pref.	AB031313
	22	Mizusawa-Gawa 2, Minehama-Mura, Akita Pref.	AB031314
	23	Komori, Takanosu-Cho, Akita Pref.	AB031315
	24	Kamisugi, Aikawa-Cho, Akita Pref.	AB031316
	25	Konda, Aikawa-Cho, Akita Pref.	AB031317
	26*	Oyu-Onsen, Kazuno-Shi, Akita Pref.	AB013867
	27	Hanaki-Dam, Takko-Cho, Aomori Pref.	AB031318
	28*	Senboku-Cho, Akita Pref.	AB013868
	29*	Shizukuishi-Cho, Iwate Pref.	AB013869
	30*	Morioka-Shi, Iwate Pref.	AB013870
D. b. babaianus	31*	Murayama-Shi, Yamagata Pref.	AB013931
	32*	Soma-Shi, Fukushima Pref.	AB013932
	33*	Kawanishi-Cho, Yamagata Pref.	AB013933
	34*	Murakami-Shi, Niigata Pref.	AB013934
D. b. oxuroides	35*	Noda-Shi, Chiba Pref.	AB013945
D. b. oxuroides	36*	Nagoya-Shi, Aichi Pref.	AB013954
D. b. blaptoides	37*	Sakurai-Shi, Nara Pref.	AB013962
D. b. brevicaudus	38*	Oki Island, Shimane Pref.	AB013973
D. b. blaptoides	39*	Yakushima Island, Kagoshima Pref.	AB013981

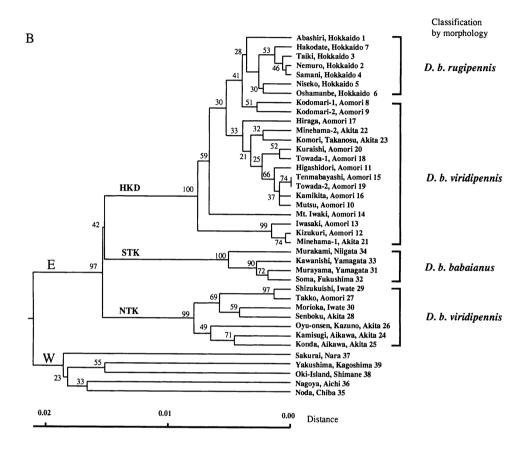
<sup>\*</sup> Taken from Su et al., 1996 b, 1998.



#### **Results and Discussion**

Figure 2 shows the ND5 phylogenetic trees for the specimens analyzed, together with those reported by SU *et al.* (1996 b, 1998). As reported in the previous study (SU *et al.*, 1998), two major lineages, the eastern (E) and the western (W), were recognized both in the NJ- and UPGMA-trees. The E lineage was further divided into three (HKD, NTK and STK) and the W lineage into five (Fig. 1) geographically linked sublineages (or races). HKD, NTK and STK in the E lineage were supported by high bootstrap values. They started to diversify almost simultaneously.

The main aim of this study is to settle the distributional boundary between HKD and NTK. As shown in Figs. 1 and 2, HKD consists of specimens from northern Akita Prefecture (loc. nos. 21–23) and those from all over Aomori Prefecture (except No. 27) as well as the *D. b. rugipennis* specimens from Hokkaido. The other three specimens (Nos. 24–26) from northern Akita and one specimen (No. 27) from southern Aomori belong to NTK. These results point to the existence of a boundary between HKD and NTK, running along the Yoneshiro-gawa River in northern Akita and the Mabechigawa River in southern Aomori (Y–M line). However, the Y–M line does not necessa-



rily mean that the common ancestor once inhabiting one region was divided into the two races by the river barriers. Perhaps the archipelago formation of the proto-Japanese Islands about 13 million years ago resulted in isolation of proto-HKD and proto-NTK in the separate islands. Following this, the two races expanded their distributions upon an extensive upheaval of the Tôhoku district until they were interrupted by the rivers. The phylogenetic trees show that HKD diversified rather anciently into several clades. The subspecies, *rugipennis* belongs to one of the clades (Fig. 2). It is notable that two specimens of *viridipennis* (loc. nos. 8 and 9) from Kodomari on the Tsugaru Peninsula were closely related to *rugipennis* on the tree (Fig. 2), suggesting that the ancestor inhabiting the northern edge of the Tsugaru Peninsula immigrated to Hokkaido through a land bridge, followed by isolation of the Hokkaido population from the

Fig. 2 (on pp. 638–639). Phylogenetic trees of the mitochondrial ND5 gene (1,069 bp upstream from 3' terminal stop codon) of *Damaster blaptoides*. The NJ tree (A) and the UPGMA tree (B). The bootstrap confidence level (%) (based on 500 resamplings) is shown at each branching point. Distance denotes Kimura's two-parameter evolutionary distance (Kimura, 1980). Not all the known sequences for STK and the W lineage are included in the trees. For details, see Su *et al.* (1998).

Tsugaru population upon formation of the Tsugaru Straits. The population of the Shimokita Peninsula would not have directly participated in this immigration because the specimens from there (loc. nos. 10 and 11) are rather remote from *rugipennis* from Hokkaido on the trees (Fig. 2).

Taxonomic notes. The type locality of rugipennis is Hakodate (Des environs de Khokodady), Hokkaido (Adams, 1861), while that of viridipennis is Aomori (Awomori; probably Aoni and the Tsugaru Peninsula) (Lewis, 1880). Since rugipennis and viridipennis in HDK are most probably consubspecific, the latter should be treated as rugipennis. Damaster b. viridipennis in NTK is phylogenetically distinct from viridipennis in HKD, and should be named after careful morphological reexamination.

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#### 要 約

金 衝坤·斉藤秀生·冨永 修·蘇 智慧·大澤省三:ミトコンドリアND5遺伝子による日 本列島東北部におけるマイマイカブリの"亜種"の分布境界線. —— これまでの形態の研究 では、エゾマイマイカブリは北海道だけに、キタカブリは東北地方の青森県・秋田県・岩手県 に分布し、2 亜種の境界線は津軽海峡であるとされてきた. Suら(1998)による分子系統解析 の結果では、東北地方の北部に分布しているキタカブリといわれたものはエゾマイマイカブリ と同一系統 (HKD) に属し、エゾマイマイと区別できず、またそれより南部のこれまでキタカブ リとされていたものは別系統 (NTK) であることが示された. したがって, 津軽海峡はマイマイ カブリ亜種の分布境界としての意味をもたないことになり, むしろ HKDと NTK の境界が重要 となる。しかし、この地域のサンプル数が不足していたため、正確な境界線が確定できなかっ た、本研究では、Suら(前述)の研究からみて分布境界線であろうと思われる青森県と秋田 県/岩手県境を中心に、多数のサンプルのミトコンドリア DNA の分析を行った、その結果、 HKDとNTK の分布境界線は、西は秋田県北部の米代川から、東は青森県南部の馬淵川から始 まり、ほぼ東西2つの川を境界に、北にはHKD、南にはNTKの分布していることがわかった。 本研究で得られた系統樹をみると、より古く青森県の西側に孤立していた集団が、陸地の隆起 とともに分布域を広げ、津軽半島を経由して北海道へ進出し、現在のエゾマイマイカブリとな ったと考えられる.

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# The Intertidal Beetle *Amblopusa magna* ZERCHE (Coleoptera, Staphylinidae, Aleocharinae) New to Hokkaido and the Kuril Archipelago

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The intertidal liparocephaline beetle, *Amblopusa magna*, was described and characterized by Zerche (1998) from the Pacific coast of Russia. Ahn and Ashe (1996) and Zerche (1998)