

板橋区役所

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塩基配列解析業務委託結果報告書

発注 No: 6310PA-IBS01



平成 26 年 10 月 15 日

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<作業内容>

作業概要として以下を行った。

1. DNA 抽出：提供されたホタル由来組織からゲノム DNA 抽出
2. PCR：文献(Suzuki ea al. Molecular Phylogenetics and Evolution, Vol. 22, No. 2, 193-205, 2002) に記載条件で PCR
3. 塩基配列解析：PCR 産物のダイレクトシーケンス
4. 相同性解析：得られたシーケンスの BLAST 解析
5. 系統樹解析：得られたシーケンスと参照配列の系統樹解析

※BLASTは、NCBIで開発された相同性検索プログラムであり、バイオインフォマティクスで最も広く使われているプログラムの一つである。

1. 供与サンプル

サンプル名	Takara ID	
せせらぎ・上陸水槽・オス1	IBS01_01	ゲンジ
せせらぎ・上陸水槽・オス2	IBS01_02	
せせらぎ・上陸水槽・オス3	IBS01_03	
せせらぎ・上陸水槽・オス4	IBS01_04	
せせらぎ・上陸水槽・オス5	IBS01_05	
せせらぎ・上陸水槽・オス6	IBS01_06	
せせらぎ・オス	IBS01_07	
せせらぎ・メス1	IBS01_08	
せせらぎ・メス2	IBS01_09	
せせらぎ・メス3	IBS01_10	
ビオトープ・オス	IBS01_11	
ビオトープ・メス	IBS01_12	
上陸水槽・メス	IBS01_13	
せせらぎ・生態水槽・オス1	IBS01_14	〜イケ
せせらぎ・生態水槽・オス2	IBS01_15	
せせらぎ・生態水槽・オス3	IBS01_16	
せせらぎ・メス	IBS01_17	
ビオトープ・オス1	IBS01_18	
ビオトープ・メス1	IBS01_19	
生態水槽・オス	IBS01_20	
生態水槽・幼虫	IBS01_21	
上陸水槽・オス	IBS01_22	
上陸水槽・メス	IBS01_23	
ビオトープ・オス2	IBS01_24	
ビオトープ・メス2	IBS01_25	

2. DNA 抽出

提供サンプルをバイオマッシャーで破碎した後に、Nucleo Spin Tissue Kit (code:740952)を用いてプロコールに準じてDNA抽出を行った。

抽出の結果、それぞれの検体で50 uLのDNA溶液を得た。DNA溶液をNanoDropを用いて測定を行ったところ、検出限界以下の低濃度であったがPCRに利用可能と判断した。

3. PCR 増幅

提供ゲノムDNAを鋳型とし、PCR反応を行った。鋳型DNAが低濃度であったため、Template DNAとして5uL使用することとした。

○ 反応組成：

Tks Gflex® DNA Polymerase

2 × Gflex PCR Buffer (Mg2+, dNTP plus)	25uL
Tks Gflex DNA Polymerase (1.25 units/uL)	1uL
2.5pmol/uL T12-J-3037 primer	4uL
2.5pmol/uL TK-N-3785 primer	4uL
Template DNA	5uL
dH2O up to	50uL

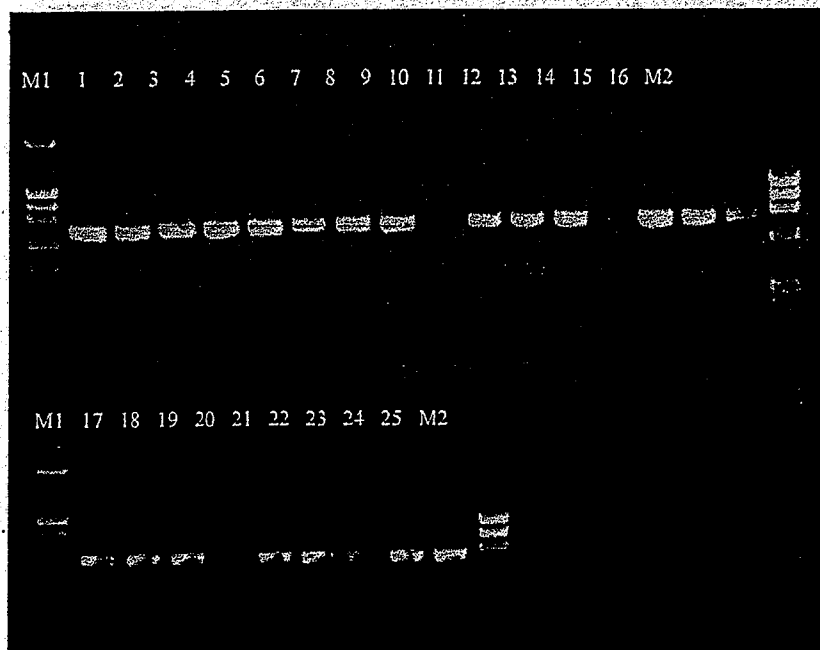
○ サーマル条件

使用機器 : TaKaRa PCR Thermal Cycler Dice® Standard

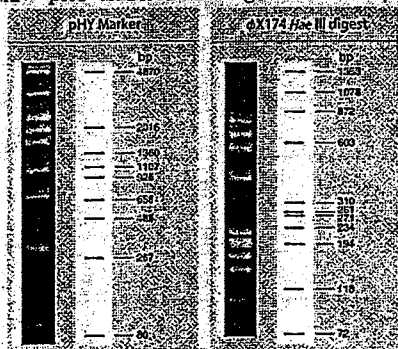
増幅条件 : 94°C 60sec.

98°C 10sec. }
50°C 15sec. } 30サイクル
68°C 60sec. }

2% アガロースゲル (Agarose L03「TAKARA」) を使用し、電気泳動で増幅確認を行った。
 (PCR 反応溶液 1 μ l を泳動)



M1: pHY Marker 100ng M2: ϕ X174/HaeIII 100ng

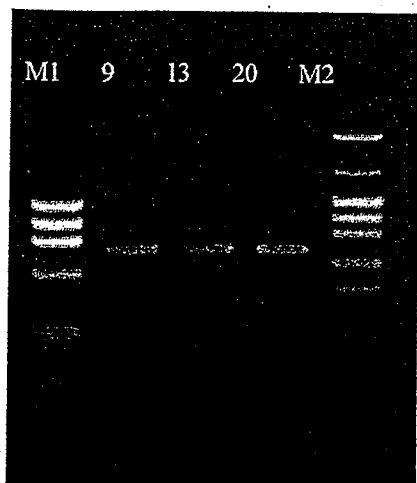


No.	サンプル名称
1	IBS01_01
2	IBS01_02
3	IBS01_03
4	IBS01_04
5	IBS01_05
6	IBS01_06
7	IBS01_07
8	IBS01_08
9	IBS01_09
10	IBS01_10
11	IBS01_11
12	IBS01_12
13	IBS01_13

no.	サンプル名称
14	IBS01_14
15	IBS01_15
16	IBS01_16
17	IBS01_17
18	IBS01_18
19	IBS01_19
20	IBS01_20
21	IBS01_21
22	IBS01_22
23	IBS01_23
24	IBS01_24
25	IBS01_25

IBS01_09、IBS01_13、IBS01_20の3検体については1度目のPCRで増幅物が得られなかったため、2度目のPCR反応を行った。PCR反応の条件は1度目の同じ条件で行った。

PCR反応後、電気泳動で増幅確認を行った。



M1: ϕ X174/HaeIII 100ng

M2: pHY Marker 100ng

2度のPCRの結果、全ての検体でPCR増幅が確認された。

4. ABI3730xlによるシーケンス解析

PCR産物をアガロースゲル電気泳動後、酵素処理により簡易精製した。これをシーケンスの鋳型として、PCRプライマーを用いてDye Terminator法によりシーケンス反応を行った (BigDye Terminator v3.1 Cycle Sequencing Kit)。

シーケンス反応後の精製はゲルろ過法 (Sephadex G-50 Fine) を用いた。

5. ベースコール

得られた波形データについてシーケンサー付属のベースコーラを用いて波形データに変換し、Quality値を得た。シーケンス名は、「サンプル名_プライマー名」とした。データを¥CD¥SEQUENCEに保存した。

6. アセンブル

SEQUENCHERソフトに、得られた波形データをimportし、シーケンス配列の前後の低QV (Quality Value) を削除後し、残った配列を有効リードとした。

有効リード (FリードおよびRリード) をアセンブルしコンティグ配列を作成した。データを¥CD¥CONTIGに保存した。

IBS01_09及びIBS01_13ではT12-J-3037プライマーでの解析で有効リードが得られなかったため、コンティグ配列ではなくTK-N-3785プライマーでの解析結果のみを保存している。

7. 相同性検索

得られたコンテイング配列をQueryとして相同性検索を行った。

- ・相同性検索の手法はBLASTを用いた。
- ・最新のGenBank Non-redundantデータベース（公共DB）に対し、Blastn検索を行った。
- ・Blastnにより得られた結果を以下の表として示す。Scoreが上位10位にランクされたものを表示した。
- ・全ての結果は電子ファイルを参照のこと。（¥CD¥BLAST¥nt-*）。表の詳細は本報告書の末尾を参照のこと。
- ・IBS01_09及びIBS01_13以外については、上位ヒット10位全てのExpect値が0であり、いずれのレコードもQueryとの相同性が非常に高いことを示す。

Rank	Query	Hit	Comment	Score	Expect	Length	QUERY SIZE
1	IBS01_01	1	dbj AB051176.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1298	0	655	655
1	IBS01_01	2	dbj AB051175.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1275	0	652	655
1	IBS01_01	3	dbj AB051174.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1267	0	651	655
1	IBS01_01	4	dbj AB051182.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1259	0	650	655
1	IBS01_01	5	dbj AB051180.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1259	0	650	655
1	IBS01_01	6	dbj AB051181.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is.	1251	0	649	655
1	IBS01_01	7	dbj AB051177.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1243	0	648	655
1	IBS01_01	8	dbj AB051178.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1243	0	648	655
1	IBS01_01	9	dbj AB051179.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1235	0	647	655
1	IBS01_01	10	dbj AB051170.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1164	0	638	655
2	IBS01_02	1	dbj AB051176.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1271	0	641	641
2	IBS01_02	2	dbj AB051175.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1247	0	638	641
2	IBS01_02	3	dbj AB051174.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1239	0	637	641

			oxidase II.complete cds. haplotype: E. from Hamasaka				
2	IBS01_02	4	dbj AB051182.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1231	0	636	641
2	IBS01_02	5	dbj AB051180.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1231	0	636	641
2	IBS01_02	6	dbj AB051181.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1223	0	635	641
2	IBS01_02	7	dbj AB051177.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1215	0	634	641
2	IBS01_02	8	dbj AB051178.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1215	0	634	641
2	IBS01_02	9	dbj AB051179.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1207	0	633	641
2	IBS01_02	10	dbj AB051170.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1136	0	624	641
3	IBS01_03	1	dbj AB051176.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1265	0	638	638
3	IBS01_03	2	dbj AB051175.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1241	0	635	638
3	IBS01_03	3	dbj AB051174.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1233	0	634	638
3	IBS01_03	4	dbj AB051182.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1225	0	633	638
3	IBS01_03	5	dbj AB051180.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1225	0	633	638
3	IBS01_03	6	dbj AB051181.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1217	0	632	638
3	IBS01_03	7	dbj AB051177.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1209	0	631	638
3	IBS01_03	8	dbj AB051178.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1209	0	631	638
3	IBS01_03	9	dbj AB051179.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1201	0	630	638
3	IBS01_03	10	dbj AB051170.1 Luciola cruciata mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1130	0	621	638

4	IBS01_04	1	dbj AB051175.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1263	0	649	653
4	IBS01_04	2	dbj AB051182.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1247	0	647	653
4	IBS01_04	3	dbj AB051181.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1239	0	646	653
4	IBS01_04	4	dbj AB051176.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1239	0	646	653
4	IBS01_04	5	dbj AB051174.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1239	0	646	653
4	IBS01_04	6	dbj AB051180.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1231	0	645	653
4	IBS01_04	7	dbj AB051177.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1231	0	645	653
4	IBS01_04	8	dbj AB051178.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1231	0	645	653
4	IBS01_04	9	dbj AB051179.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1223	0	644	653
4	IBS01_04	10	dbj AB051170.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1152	0	635	653
5	IBS01_05	1	dbj AB051176.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1283	0	647	647
5	IBS01_05	2	dbj AB051175.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1259	0	644	647
5	IBS01_05	3	dbj AB051174.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1251	0	643	647
5	IBS01_05	4	dbj AB051182.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1243	0	642	647
5	IBS01_05	5	dbj AB051180.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1243	0	642	647
5	IBS01_05	6	dbj AB051181.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1235	0	641	647
5	IBS01_05	7	dbj AB051177.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1227	0	640	647
5	IBS01_05	8	dbj AB051178.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1227	0	640	647

			oxidase II.complete cds. haplotype: F. from Numazu				
5	IBS01_05	9	dbj AB051179.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1219	0	639	647
5	IBS01_05	10	dbj AB051170.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1148	0	630	647
6	IBS01_06	1	dbj AB051175.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1235	0	641	647
6	IBS01_06	2	dbj AB051182.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1219	0	639	647
6	IBS01_06	3	dbj AB051181.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1211	0	638	647
6	IBS01_06	4	dbj AB051176.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1211	0	638	647
6	IBS01_06	5	dbj AB051174.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1211	0	638	647
6	IBS01_06	6	dbj AB051180.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1203	0	637	647
6	IBS01_06	7	dbj AB051177.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1203	0	637	647
6	IBS01_06	8	dbj AB051178.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1203	0	637	647
6	IBS01_06	9	dbj AB051179.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1195	0	636	647
6	IBS01_06	10	dbj AB051170.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1124	0	627	647
7	IBS01_07	1	dbj AB051176.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1277	0	650	652
7	IBS01_07	2	dbj AB051175.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1253	0	647	652
7	IBS01_07	3	dbj AB051174.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1245	0	646	652
7	IBS01_07	4	dbj AB051182.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1237	0	645	652
7	IBS01_07	5	dbj AB051180.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1237	0	645	652

7	IBS01_07	6	dbj AB051181.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1229	0	644	652
7	IBS01_07	7	dbj AB051177.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1221	0	643	652
7	IBS01_07	8	dbj AB051178.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1221	0	643	652
7	IBS01_07	9	dbj AB051179.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1213	0	642	652
7	IBS01_07	10	dbj AB051170.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1142	0	633	652
8	IBS01_08	1	dbj AB051175.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1247	0	641	645
8	IBS01_08	2	dbj AB051182.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1231	0	639	645
8	IBS01_08	3	dbj AB051181.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1223	0	638	645
8	IBS01_08	4	dbj AB051176.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1223	0	638	645
8	IBS01_08	5	dbj AB051174.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1223	0	638	645
8	IBS01_08	6	dbj AB051180.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1215	0	637	645
8	IBS01_08	7	dbj AB051177.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1215	0	637	645
8	IBS01_08	8	dbj AB051178.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1215	0	637	645
8	IBS01_08	9	dbj AB051179.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1207	0	636	645
8	IBS01_08	10	dbj AB051170.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1136	0	627	645
9	IBS01_09 TK-N-37 85	1	dbj AB080702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Chiba, Ohara	396	1E-10 7	217	232
9	IBS01_09 TK-N-37	2	dbj AB080713.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Nagasaki,Tsushima Is	388	1E-10 4	216	232

85							
9	IBS01_09_ TK-N-37 85	3	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Nagano,Matsumoto	388	1E-10 4	216	232
9	IBS01_09_ TK-N-37 85	4	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Shizuoka, Izu	388	1E-10 4	216	232
9	IBS01_09_ TK-N-37 85	5	dbj AB080709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamaguchi,Yamaguchi	388	1E-10 4	216	232
9	IBS01_09_ TK-N-37 85	6	dbj AB080708.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan: Ehime, Matsuyama	388	1E-10 4	216	232
9	IBS01_09_ TK-N-37 85	7	dbj AB080706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Fukushima, Iwaki	388	1E-10 4	216	232
9	IBS01_09_ TK-N-37 85	8	dbj AB080701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamagata,Yonezawa	388	1E-10 4	216	232
9	IBS01_09_ TK-N-37 85	9	dbj AB080700.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Hokkaido,Kushiro	388	1E-10 4	216	232
9	IBS01_09_ TK-N-37 85	10	dbj AB080718.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Kochi, Tosayama	380	1E-10 2	215	232
10	IBS01_10	1	dbj AB051176.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1279	0	651	653
10	IBS01_10	2	dbj AB051175.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1255	0	648	653
10	IBS01_10	3	dbj AB051174.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1247	0	647	653
10	IBS01_10	4	dbj AB051182.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1239	0	646	653
10	IBS01_10	5	dbj AB051180.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1239	0	646	653

10	IBS01_10	6	dbj AB051181.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1231	0	645	653
10	IBS01_10	7	dbj AB051177.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1223	0	644	653
10	IBS01_10	8	dbj AB051178.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1223	0	644	653
10	IBS01_10	9	dbj AB051179.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1215	0	643	653
10	IBS01_10	10	dbj AB051170.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1144	0	634	653
11	IBS01_11	1	dbj AB051177.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Matsumoto	1267	0	645	647
11	IBS01_11	2	dbj AB051178.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: F. from Numazu	1267	0	645	647
11	IBS01_11	3	dbj AB051179.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: G. from Minokamo	1259	0	644	647
11	IBS01_11	4	dbj AB051175.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1251	0	643	647
11	IBS01_11	5	dbj AB051176.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1243	0	642	647
11	IBS01_11	6	dbj AB051182.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: J. from Tetta	1235	0	641	647
11	IBS01_11	7	dbj AB051181.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: I. from Oki Is	1227	0	640	647
11	IBS01_11	8	dbj AB051174.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1227	0	640	647
11	IBS01_11	9	dbj AB051180.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: H. from Tsugu	1219	0	639	647
11	IBS01_11	10	dbj AB051170.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: B. from Yonezawa	1156	0	631	647
12	IBS01_12	1	dbj AB051176.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Numazu	1294	0	653	653
12	IBS01_12	2	dbj AB051175.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hiroshima	1271	0	650	653
12	IBS01_12	3	dbj AB051174.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II.complete cds. haplotype: E. from Hamasaka	1263	0	649	653

			oxidase II, complete cds, haplotype: E. from Hamasaka				
12	IBS01_12	4	dbj AB051182.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II, complete cds, haplotype: J. from Tetta	1255	0	648	653
12	IBS01_12	5	dbj AB051180.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II, complete cds, haplotype: H. from Tsugu	1255	0	648	653
12	IBS01_12	6	dbj AB051181.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II, complete cds, haplotype: I. from Oki Is	1247	0	647	653
12	IBS01_12	7	dbj AB051177.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II, complete cds, haplotype: F. from Matsumoto	1239	0	646	653
12	IBS01_12	8	dbj AB051178.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II, complete cds, haplotype: F. from Numazu	1239	0	646	653
12	IBS01_12	9	dbj AB051179.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II, complete cds, haplotype: G. from Minokamo	1231	0	645	653
12	IBS01_12	10	dbj AB051170.1 <i>Luciola cruciata</i> mitochondrial COII gene for cytochrome oxidase II, complete cds, haplotype: B. from Yonezawa	1160	0	636	653
13	IBS01_13_ TK-N-37 85	1	dbj AB080717.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidase subunit II, complete cds, country: Japan: Kagoshima, Kajiki	337	3.00E-89	198	228
13	IBS01_13_ TK-N-37 85	2	dbj AB080716.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidase subunit II, complete cds, country: Japan: Aichi, Toyohashi	337	3.00E-89	198	228
13	IBS01_13_ TK-N-37 85	3	dbj AB080715.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidase subunit II, complete cds, country: Japan: Nagano, Iizuna	337	3.00E-89	198	228
13	IBS01_13_ TK-N-37 85	4	dbj AB080713.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidase subunit II, complete cds, country: Japan: Nagasaki, Tsushima Is	337	3.00E-89	198	228
13	IBS01_13_ TK-N-37 85	5	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidase subunit II, complete cds, country: Japan: Nagano, Matsumoto	337	3.00E-89	198	228
13	IBS01_13_ TK-N-37 85	6	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidase subunit II, complete cds, country: Japan: Shizuoka, Izu	337	3.00E-89	198	228
13	IBS01_13_ TK-N-37 85	7	dbj AB080710.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidase subunit II, complete cds, country: Japan: Miyagi, Narugo	337	3.00E-89	198	228

	85						
13	IBS01_13_85	8	dbj AB080709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamaguchi.Yamaguchi	337	3.00E-89	198	228
13	IBS01_13_85	9	dbj AB080708.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Ehime. Matsuyama	337	3.00E-89	198	228
13	IBS01_13_85	10	dbj AB080706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Fukushima. Iwaki	337	3.00E-89	198	228
14	IBS01_14	1	dbj AB080716.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Aichi. Toyohashi	1239	0	639	646
14	IBS01_14	2	dbj AB080717.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Kagoshima.Kajiki	1207	0	635	646
14	IBS01_14	3	dbj AB080709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamaguchi.Yamaguchi	1207	0	635	646
14	IBS01_14	4	dbj AB080703.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Aomori. Hirosaki	1207	0	635	646
14	IBS01_14	5	dbj AB080705.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Kanagawa.Yokosuka	1207	0	635	646
14	IBS01_14	6	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Nagano.Matsumoto	1191	0	633	646
14	IBS01_14	7	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Shizuoka. Izu	1191	0	633	646
14	IBS01_14	8	dbj AB080707.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Shiga. Ibuki	1191	0	633	646
14	IBS01_14	9	dbj AB080706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Fukushima. Iwaki	1191	0	633	646
14	IBS01_14	10	dbj AB080718.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Kochi. Tosayama	1183	0	632	646
15	IBS01_15	1	dbj AB080702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Chiba. Ohara	1225	0	635	643
15	IBS01_15	2	dbj AB080701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamagata.Yonezawa	1217	0	634	643
15	IBS01_15	3	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome	1209	0	633	643

			oxidasesubunit II, complete cds, country:Japan:Nagano.Matsumoto				
226	15	IBS01_15	4 dbj AB080712.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Shizuoka, Izu	1209	0	633	643
228	15	IBS01_15	5 dbj AB080710.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Miyagi, Narugo	1209	0	633	643
228	15	IBS01_15	6 dbj AB080709.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Yamaguchi.Yamaguchi	1209	0	633	643
228	15	IBS01_15	7 dbj AB080706.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Fukushima, Iwaki	1209	0	633	643
646	15	IBS01_15	8 dbj AB080700.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Hokkaido,Kushiro	1209	0	633	643
646	15	IBS01_15	9 dbj AB080715.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Nagano, Iizuna	1201	0	632	643
646	15	IBS01_15	10 dbj AB080717.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Kagoshima,Kajiki	1193	0	631	643
646	16	IBS01_16	1 dbj AB080701.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Yamagata.Yonezawa	1219	0	635	644
646	16	IBS01_16	2 dbj AB080711.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Nagano.Matsumoto	1211	0	634	644
646	16	IBS01_16	3 dbj AB080712.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Shizuoka, Izu	1211	0	634	644
646	16	IBS01_16	4 dbj AB080710.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Miyagi, Narugo	1211	0	634	644
646	16	IBS01_16	5 dbj AB080709.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Yamaguchi.Yamaguchi	1211	0	634	644
646	16	IBS01_16	6 dbj AB080706.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Fukushima, Iwaki	1211	0	634	644
646	16	IBS01_16	7 dbj AB080702.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Chiba, Ohara	1211	0	634	644
643	16	IBS01_16	8 dbj AB080700.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Hokkaido,Kushiro	1211	0	634	644
643	16	IBS01_16	9 dbj AB080715.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Nagano, Iizuna	1203	0	633	644
643	16	IBS01_16	10 dbj AB080717.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Kagoshima,Kajiki	1195	0	632	644

17	IBS01_17	1	dbj AB080701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Yamagata.Yonezawa	1241	0	643	651
17	IBS01_17	2	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Nagano.Matsumoto	1233	0	642	651
17	IBS01_17	3	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Shizuoka.Izu	1233	0	642	651
17	IBS01_17	4	dbj AB080710.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Miyagi.Narugo	1233	0	642	651
17	IBS01_17	5	dbj AB080709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Yamaguchi.Yamaguchi	1233	0	642	651
17	IBS01_17	6	dbj AB080706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Fukushima.Iwaki	1233	0	642	651
17	IBS01_17	7	dbj AB080702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Chiba.Ohara	1233	0	642	651
17	IBS01_17	8	dbj AB080700.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Hokkaido.Kushiro	1233	0	642	651
17	IBS01_17	9	dbj AB080715.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Nagano.Iizuna	1225	0	641	651
17	IBS01_17	10	dbj AB080717.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Kagoshima.Kajiki	1217	0	640	651
18	IBS01_18	1	dbj AB080702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Chiba.Ohara	1227	0	636	644
18	IBS01_18	2	dbj AB080701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Yamagata.Yonezawa	1219	0	635	644
18	IBS01_18	3	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Nagano.Matsumoto	1211	0	634	644
18	IBS01_18	4	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Shizuoka.Izu	1211	0	634	644
18	IBS01_18	5	dbj AB080710.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Miyagi.Narugo	1211	0	634	644
18	IBS01_18	6	dbj AB080709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Yamaguchi.Yamaguchi	1211	0	634	644
18	IBS01_18	7	dbj AB080706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Fukushima.Iwaki	1211	0	634	644
18	IBS01_18	8	dbj AB080700.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan;Hokkaido.Kushiro	1211	0	634	644

			oxidasesubunit II, complete cds. country:Japan:Hokkaido,Kushiro				
18	IBS01_18	9	dbj AB080715.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano,Iizuna	1203	0	633	644
18	IBS01_18	10	dbj AB080717.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Kagoshima,Kajiki	1195	0	632	644
19	IBS01_19	1	dbj AB080702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Chiba,Ohara	1219	0	632	640
19	IBS01_19	2	dbj AB080701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamagata,Yonezawa	1211	0	631	640
19	IBS01_19	3	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano,Matsumoto	1203	0	630	640
19	IBS01_19	4	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Shizuoka,Izu	1203	0	630	640
19	IBS01_19	5	dbj AB080710.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Miyagi,Narugo	1203	0	630	640
19	IBS01_19	6	dbj AB080709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamaguchi,Yamaguchi	1203	0	630	640
19	IBS01_19	7	dbj AB080706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Fukushima,Iwaki	1203	0	630	640
19	IBS01_19	8	dbj AB080700.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Hokkaido,Kushiro	1203	0	630	640
19	IBS01_19	9	dbj AB080715.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano,Iizuna	1195	0	629	640
19	IBS01_19	10	dbj AB080717.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Kagoshima,Kajiki	1187	0	628	640
20	IBS01_20	1	dbj AB080702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Chiba,Ohara	1245	0	645	653
20	IBS01_20	2	dbj AB080701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamagata,Yonezawa	1237	0	644	653
20	IBS01_20	3	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano,Matsumoto	1229	0	643	653
20	IBS01_20	4	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Shizuoka,Izu	1229	0	643	653
20	IBS01_20	5	dbj AB080710.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Miyagi,Narugo	1229	0	643	653

20	IBS01_20	6	dbj ABO80709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamaguchi.Yamaguchi	1229	0	643	653
20	IBS01_20	7	dbj ABO80706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Fukushima.Iwaki	1229	0	643	653
20	IBS01_20	8	dbj ABO80700.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Hokkaido.Kushiro	1229	0	643	653
20	IBS01_20	9	dbj ABO80715.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Nagano.Iizuna	1221	0	642	653
20	IBS01_20	10	dbj ABO80717.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Kagoshima.Kajiki	1213	0	641	653
21	IBS01_21	1	dbj ABO80701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamagata.Yonezawa	1170	0	607	615
21	IBS01_21	2	dbj ABO80711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Nagano.Matsumoto	1162	0	606	615
21	IBS01_21	3	dbj ABO80712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Shizuoka.Izu	1162	0	606	615
21	IBS01_21	4	dbj ABO80710.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Miyagi.Narugo	1162	0	606	615
21	IBS01_21	5	dbj ABO80709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamaguchi.Yamaguchi	1162	0	606	615
21	IBS01_21	6	dbj ABO80706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Fukushima.Iwaki	1162	0	606	615
21	IBS01_21	7	dbj ABO80702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Chiba.Ohara	1162	0	606	615
21	IBS01_21	8	dbj ABO80700.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Hokkaido.Kushiro	1162	0	606	615
21	IBS01_21	9	dbj ABO80715.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Nagano.Iizuna	1154	0	605	615
21	IBS01_21	10	dbj ABO80717.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Kagoshima.Kajiki	1146	0	604	615
22	IBS01_22	1	dbj ABO80702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Chiba.Ohara	1183	0	614	622
22	IBS01_22	2	dbj ABO80701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Yamagata.Yonezawa	1176	0	613	622
22	IBS01_22	3	dbj ABO80711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II. complete cds. country:Japan:Nagano.Matsumoto	1168	0	612	622

			oxidasesubunit II, complete cds. country:Japan:Nagano:Matsumoto				
22	IBS01_22	4	dbj AB080712.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Shizuoka. Izu	1168	0	612	622
22	IBS01_22	5	dbj AB080710.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Miyagi. Narugo	1168	0	612	622
22	IBS01_22	6	dbj AB080709.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamaguchi.Yamaguchi	1168	0	612	622
22	IBS01_22	7	dbj AB080706.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Fukushima. Iwaki	1168	0	612	622
22	IBS01_22	8	dbj AB080700.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Hokkaido.Kushiro	1168	0	612	622
22	IBS01_22	9	dbj AB080715.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano. Iizuna	1160	0	611	622
22	IBS01_22	10	dbj AB080717.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Kagoshima.Kajiki	1152	0	610	622
23	IBS01_23	1	dbj AB080701.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamagata.Yonezawa	1203	0	624	632
23	IBS01_23	2	dbj AB080711.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano:Matsumoto	1195	0	623	632
23	IBS01_23	3	dbj AB080712.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Shizuoka. Izu	1195	0	623	632
23	IBS01_23	4	dbj AB080710.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Miyagi. Narugo	1195	0	623	632
23	IBS01_23	5	dbj AB080709.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamaguchi.Yamaguchi	1195	0	623	632
23	IBS01_23	6	dbj AB080706.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Fukushima. Iwaki	1195	0	623	632
23	IBS01_23	7	dbj AB080702.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Chiba. Ohara	1195	0	623	632
23	IBS01_23	8	dbj AB080700.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Hokkaido.Kushiro	1195	0	623	632
23	IBS01_23	9	dbj AB080715.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano. Iizuna	1187	0	622	632
23	IBS01_23	10	dbj AB080717.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Kagoshima.Kajiki	1179	0	621	632

24	IBS01_24	1	dbj AB080701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamagata.Yonezawa	1229	0	640	649
24	IBS01_24	2	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano.Matsumoto	1221	0	639	649
24	IBS01_24	3	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Shizuoka.Izu	1221	0	639	649
24	IBS01_24	4	dbj AB080710.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Miyagi.Narugo	1221	0	639	649
24	IBS01_24	5	dbj AB080709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamaguchi.Yamaguchi	1221	0	639	649
24	IBS01_24	6	dbj AB080706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Fukushima.Iwaki	1221	0	639	649
24	IBS01_24	7	dbj AB080702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Chiba.Ohara	1221	0	639	649
24	IBS01_24	8	dbj AB080700.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Hokkaido.Kushiro	1221	0	639	649
24	IBS01_24	9	dbj AB080715.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano.Iizuna	1213	0	638	649
24	IBS01_24	10	dbj AB080717.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Kagoshima.Kajiki	1205	0	637	649
25	IBS01_25	1	dbj AB080701.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamagata.Yonezawa	1239	0	642	650
25	IBS01_25	2	dbj AB080711.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Nagano.Matsumoto	1231	0	641	650
25	IBS01_25	3	dbj AB080712.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Shizuoka.Izu	1231	0	641	650
25	IBS01_25	4	dbj AB080710.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Miyagi.Narugo	1231	0	641	650
25	IBS01_25	5	dbj AB080709.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Yamaguchi.Yamaguchi	1231	0	641	650
25	IBS01_25	6	dbj AB080706.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Fukushima.Iwaki	1231	0	641	650
25	IBS01_25	7	dbj AB080702.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Chiba.Ohara	1231	0	641	650
25	IBS01_25	8	dbj AB080700.1 <i>Luciola lateralis</i> mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds. country:Japan:Hokkaido.Kushiro	1231	0	641	650

			oxidasesubunit II, complete cds, country:Japan:Hokkaido:Kushiro				
25	IBS01_25	9	dbj AB080715.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Nagano,iizuna	1223	0	640	650
25	IBS01_25	10	dbj AB080717.1 Luciola lateralis mitochondrial CO II gene for cytochrome oxidasesubunit II, complete cds, country:Japan:Kagoshima:Kajiki	1215	0	639	650

※相同性検索でヒットしたレコードは全てヘイケボタルもしくはゲンジボタルのミトコンドリアのシトクロームオキシダーゼII遺伝子であった。

8. 系統樹作成結果

- ・解析用ソフトMEGA (MOLECULAR EVOLUTIONARY GENETICS ANALYSIS) を使用して系統樹作成を行った。
- ・解析に投入した塩基配列は、検体として、6でアセンブルした配列 (本作業で得られた配列) および参照配列として、データベースに登録されたCO2遺伝子配列である。

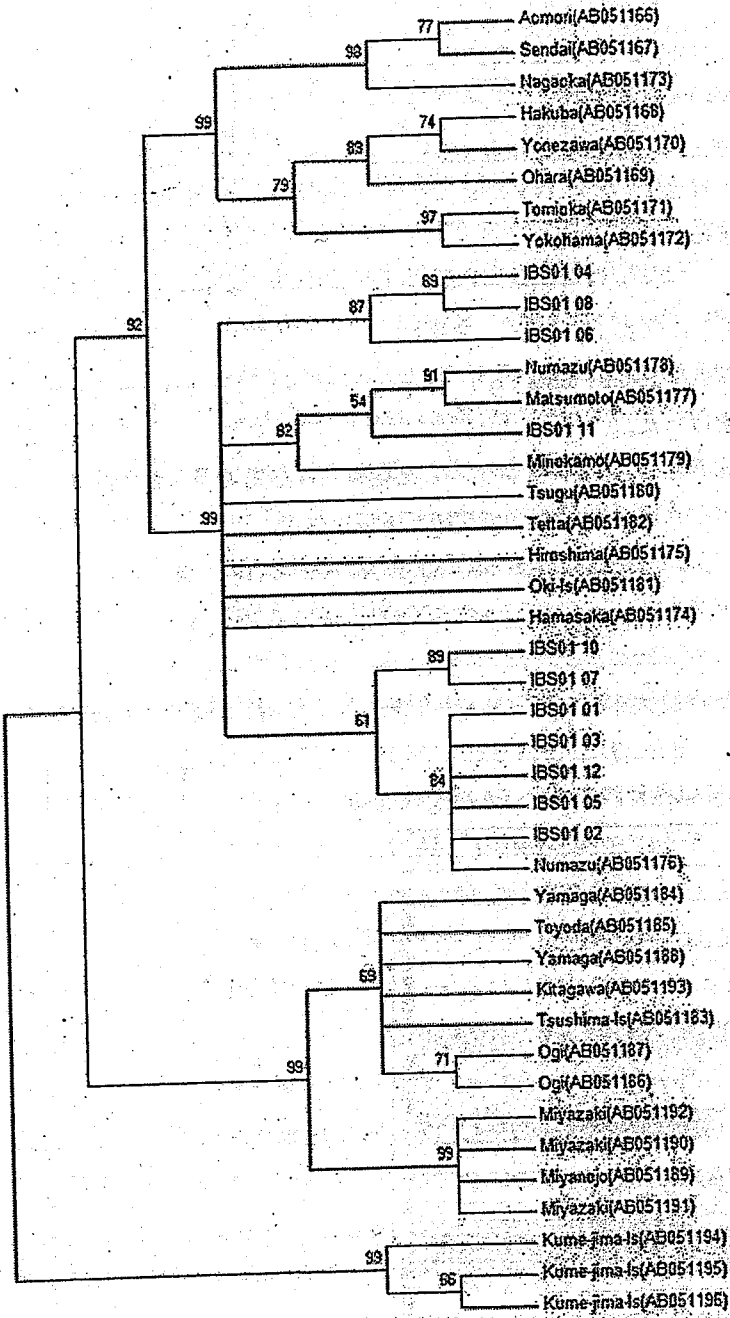
IBS01_01からIBS01_13はゲンジボタル (Accession No. AB051166-AB051196) を、
IBS01_14からIBS01_25はヘイケボタル (Accession No. AB080700-AB080720) を
参照配列とした。

- ・IBS01_09及びIBS01_13では正確な結果が得られないことが予想されるため、系統樹から除外した。
- ・系統樹作成はUPGMA法を用いた。詳細条件設定は以下の条件である。

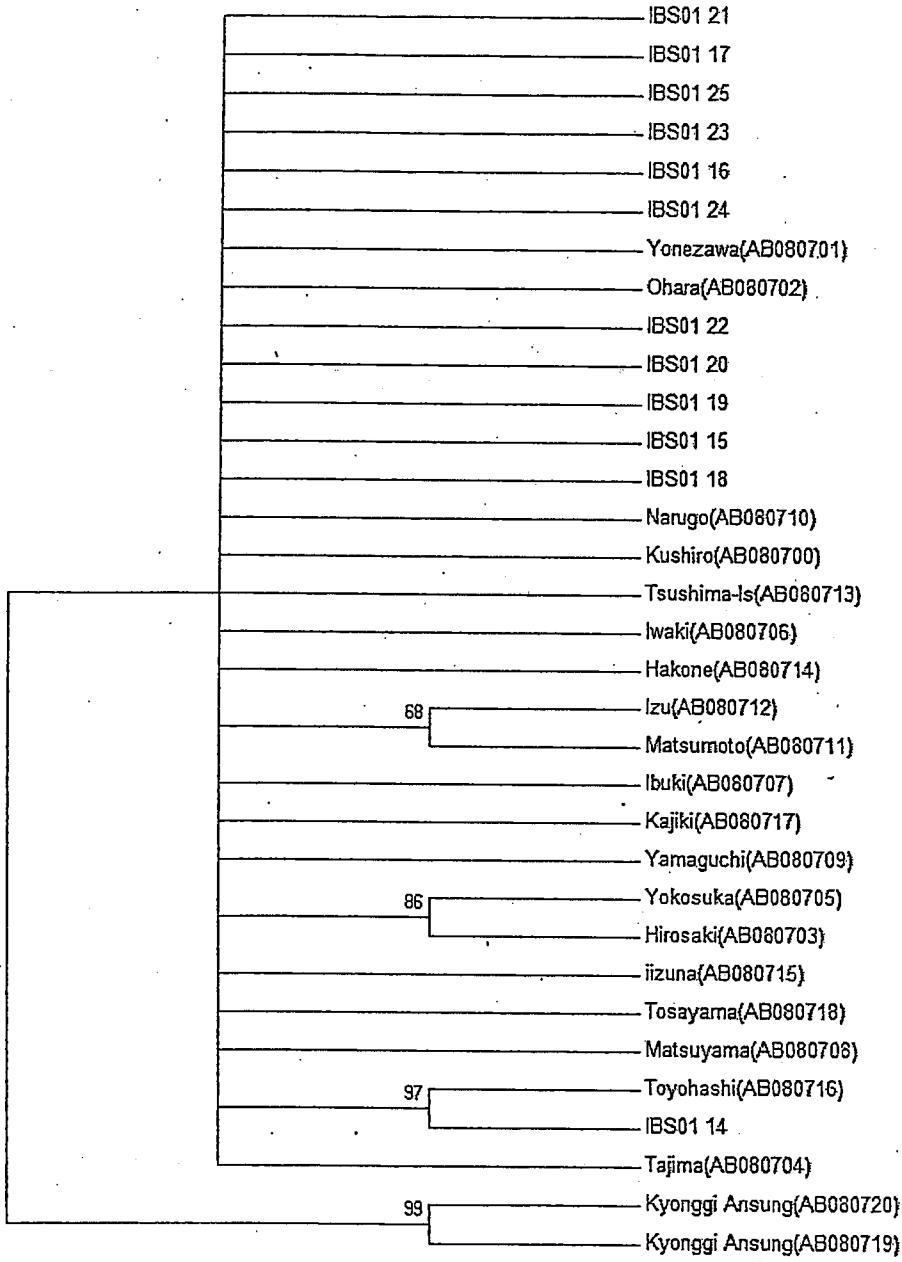
Option	Selection
Analysis	Phylogeny Reconstruction
Scope	All Selected Taxa
Statistical Method	UPGMA
Phylogeny Test	
Test of Phylogeny	Bootstrap method
No. of Bootstrap Replications	500
Substitution Model	
Substitutions Type	Nucleotide
Model/Method	Maximum Composite Likelihood
Substitutions to Include	d1 Transitions + Transversions
Rates and Patterns	
Rates among Sites	Uniform rates
Gamma Parameter	Not Applicable
Pattern among Lineages	Same (Homogeneous)
Data Subset to Use	
Gaps/Missing Data Treatment	Complete deletion
Use Consensus Method (%)	Not Applicable

- ・得られた系統樹を次のページに示した。電子ファイルとして、同じものをCDに格納した。
- 保存先: ¥CD¥CUSTOM

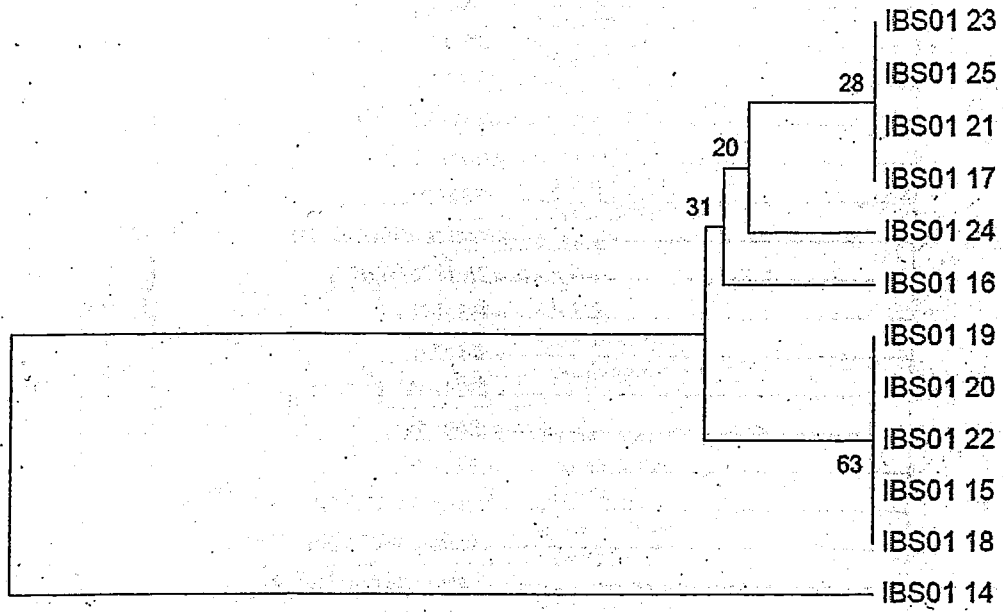
・ゲンジボタル



・ヘイケボタル



・ヘイケボタル (検体のみ)



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○「ゲンジボタルにみる遺伝的多様性・固有性と人為的攪乱の状況（鈴木浩文）」にて報告されている系統樹と、本試験でのゲンジボタルの系統樹作成の結果を比較した。

- ・ IBS01_11はゲンジボタルにおける西日本系統グループⅢと最も相同性が高い。
- ・ IBS01_01, 02, 03, 05, 07, 10, 12はゲンジボタルの西日本系統グループⅣと最も相同性が高い。
- ・ IBS01_04, 06, 08, はゲンジボタルの西日本系統と最も相同性が高い。

○「ホタルの系統と進化-ミトコンドリアDNAからのアプローチ（鈴木浩文）」にて報告されている系統樹と、本試験でのヘイケボタルの系統樹作成の結果を比較した。

- ・ IBS01_14は、Toyohashi (AB080716) 配列との相同性が最も高い。
- ・ その他の検体については、Kyonggi

Ansung (AB080719, AB080720) を除く参照配列全てと同程度の相同性を認めた。

- ・ ヘイケボタル由来サンプル12検体 (IBS01_14

～IBS01_25) を系統樹解析した場合、1) IBS01_14のみ、2) IBS01_14以外の11検体という2つにグループ分けされる。このうち、2) IBS01_14以外の11検体は相互に相同性が非常に高い。

ヘイケボタルの検体同士のアライメント図（配列比較図）を次ページに掲載する。

尚、比較対象とした系統樹を参考として以下に示す。

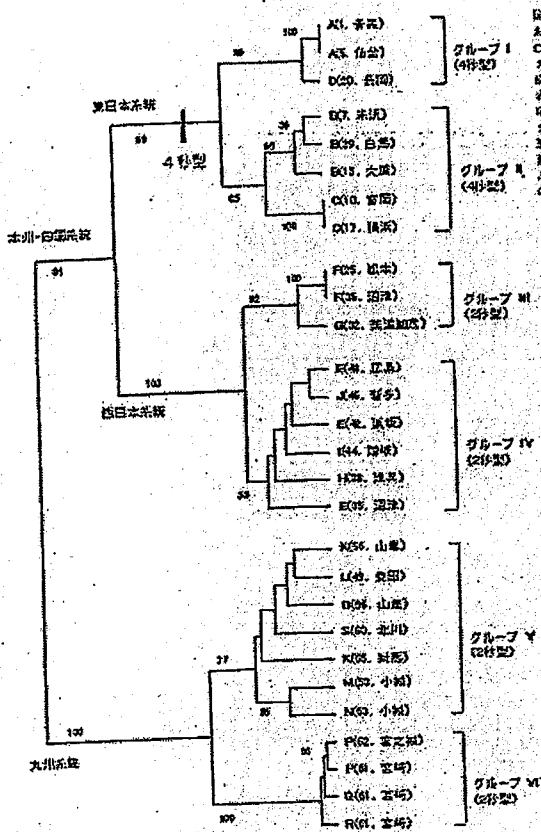
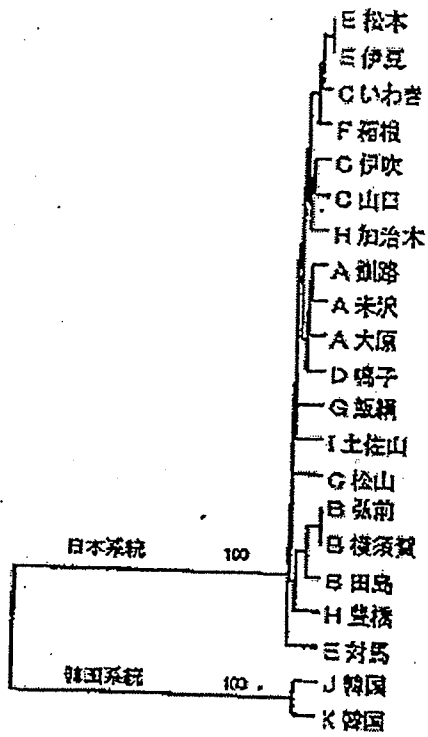


図2 ゲンジボタルにおけるミトコンドリアCOII遺伝子のハプロタイプ(A-S)の系統樹。加註とその番号は表2の範囲内番号に対応する。I-VIのハプロタイプ・グループの発生順序推定は、本図が提示した推定を記している。以下のスケールは1%の塩基置換。

CO2遺伝子から作成したゲンジボタルの系統樹

出典:「ゲンジボタルにみる遺伝的多様性・固有性と人為的撹乱の状況(鈴木浩文:月刊海洋/号外 No.51,2009)」



CO2遺伝子から作成したヘイケボタルの系統樹

出典: ボタルの系統と進化-ミトコンドリアDNAからのアプローチ (鈴木浩文: 昆虫と自然39(8).2004)

以上

<納品物>

- 作業報告書 (本紙) 1部
- CD 1枚

以下に納品CDデータ内容を示す。

フォルダ名	内容
SEQUENCE	.ab1 : シークエンサー付属のベースコーラを用いて波形配列データに変換したABI形式クロマトデータ。 .txt : シークエンサー付属のベースコーラを用いて波形配列データに変換したplain塩基配列テキストデータ
CONTIG	アセンブルして作成したコンティグ配列 (.TXT) アセンブルデータ (.SPF) : SEQUENCHERソフトで編集したデータ。 ※SEQUENCHER_demoをインストールすることで閲覧可能
CUSTOM	.meg : 解析ソフトMEGAで作成したアライメントデータ .mts : 解析ソフトMEGAで出力した系統樹データ .png : 作成した系統樹を画像ファイルで出力したもの .pdf : 作成した系統樹をpdf形式で出力したもの
SEQUENCHER_demo	波形閲覧ソフト

波形データを閲覧するには波形閲覧ソフトが必要となります。

- Sequence Scanner ソフトウェア ver1.0 -

このソフトは波形の生データとベースコールデータの閲覧が出来ます。

波形の印刷が可能です。

Sequence Scanner ソフトウェア ver1.0ダウンロードアドレス

<https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&atID=600583&tab=DetailInfo>

- SEQUENCHER -

このソフトは波形閲覧とアセンブルを両方できる機能を備えています。

SEQUENCHERのdemo版でしたら無償でインターネットからダウンロード可能です。

ただしdemo版ですので印刷や保存が出来ません。

SEQUENCHER demo版ダウンロードアドレス

<http://www.genecodes.com/> ダウンロード頁へ

SEQUENCHER最新バージョン (製品コード

9261、9260) は弊社でご購入いただけます。

http://catalog.takara-bio.co.jp/product/basic_info.php?catcd=B1000475&subcatcd=B1000480&unitid=U100003596

<相同性検索について> (**-blastn.csv)

blast 検索結果を csv 形式(カンマ区切りのテキストファイル)で格納している。
Microsoft EXCEL やテキストエディターで閲覧することが可能。各カラムの説明は下表を参照のこと。

■No	Hit(ヒット)毎につく連番
■QueryNo	各 query につく番号
■Query	検索に用いた配列名
■QUERY_SIZE	query の配列長
■Database	BLAST 検索に用いたデータベース
■QUERY_HIT	1 つの query 内で複数ヒットした場合にクエリ内で行く連番 top hit は1となる
■Comment	ヒットした配列の ID
■ ORG	Comment より生物種名を抽出(ただしコンピュータで機械的に抽出しているため実際は種名でない場合も含まれる)
■ Kind	BLASTn の場合: Comment より「16S」「18S」「chromosome」「mRNA」「cDNA」「STS」「Vector」「gene」のキーワードを抽出 BLASTx の場合: Comment より「ase」「hypothetical」「inhibitor」「ribosomal」「receptor」「subunit」のキーワードを抽出
■Comment2	ヒットした配列の Accession ID
■Length	ヒットした配列の配列長
■HitPosNo	query が 1 つの subject の複数個所にヒットした場合に subject の位置 毎に付与される連番 (微生物推定解析の場合にはすべて 1 と表示)
■Score	検索結果の相同性スコア
■Expect	検索結果の期待値
■Identities_1	同一残基(塩基)数 一致した塩基数
■Identities_2	同一残基(塩基)数 アライメントされた塩基数
■Positives_1	アミノ酸の場合のみ表示 同一残基数 一致したアミノ酸残基数
■Positives_2	アミノ酸の場合のみ表示 同一残基数 アライメントされたアミノ酸残基数
■Frame	翻訳のフレーム
■Gaps	アライメント中でギャップとなった残基数
■Strand	query と subject のアライメントの際の方向
■QUERY_START	query 上でのアライメントの開始位置
■QUERY_END	query 上でのアライメントの終了位置
■SUBJECT_START	subject 上でのアライメントの開始位置
■SUBJECT_END	subject 上でのアライメントの終了位置
■QUERY_STRING	アライメントされた部位の query の配列
■SUBJECT_STRING	アライメントされた部位の subject の配列
■Linkadless	ヒットした subject のリンク情報(URL) *
■P	空欄

* blastn(x).csv ファイルを開けて別名保存をすると、リンク情報が失われます。ファイルは閉じた状態でファイルごとコピーを行ってください。リンク情報は NCBI の相同性検索のみです。

・BLAST-NCBI 検索についての詳細は、<http://www.ncbi.nlm.nih.gov/BLAST/>