Morphometric and Molecular identification of the female castes of *Bombus ignitus* and *B. ardens* (Apidae: Hymenoptera)

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Introduction

Background. Bumblebees are important pollinators for wildflowers and agricultural crops. Identification of bumblebees based on morphological characters is difficult, especially for non-experts, due to lack of the reliable characters and presence of intraspecific variation in colour patterns. Among Korean bumblebees, *Bombus ignitus* and *B. ardens* are relatively abundant and important for pollination of wildflowers and agricultural crops. Although the males are easily distinguishable phenotypically, the female castes are difficult to identify from each other (Fig. 1).

Aims. The aim of this study is to find the rapid method in identification of these important pollinators using morphological and molecular traits.

Material and Methods

Materials. 35 previously-known samples from each species, identified by DNA barcoding were used in morphometric analysis.
Methods. Twelve morphometric traits were analyzed to evaluate their strength in precise identification of these two species (Fig. 2). In addition, PCR–RFLP method was used for identification of these two bumblebee species. Partial mitochondrial COI fragment (435 bp) and three diagnostic restriction enzymes (AluI, BspHI and EarI) were used to identify species from degraded DNA material.

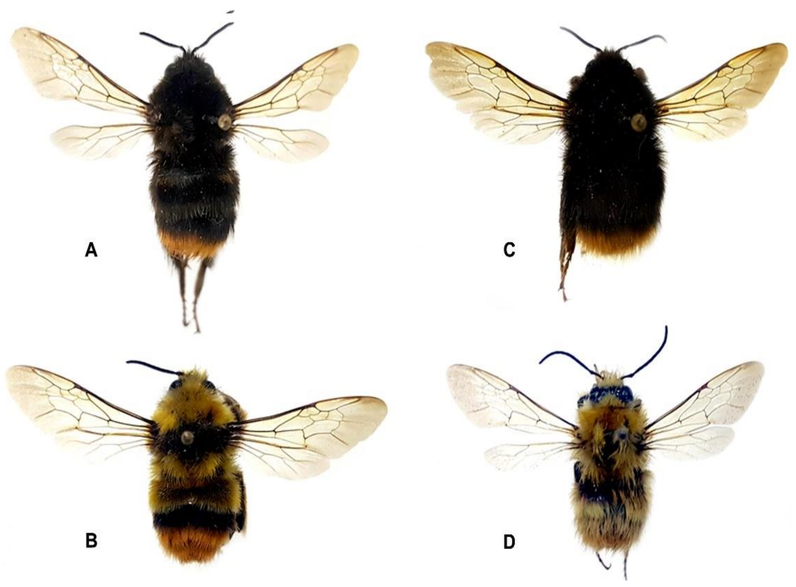


Figure 1. *Bombus* spp. *B. ignitus* (A, B); *B. ardens* (C, D); queen (A, C); drone (B, D)

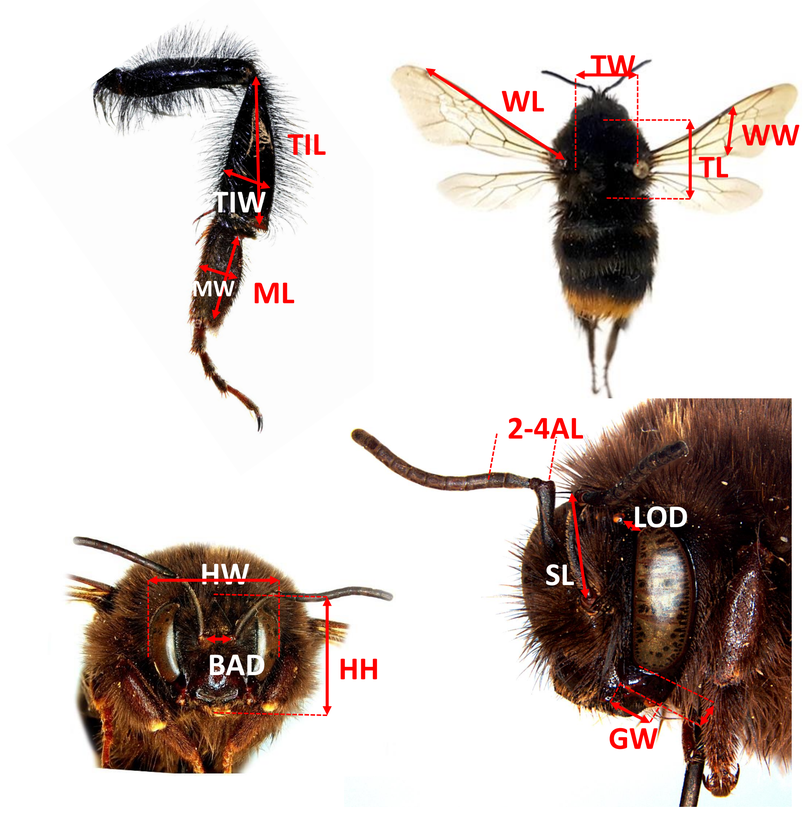


Figure 2. The morphometric characters used in identification of *B. ignitus* and *B. ardens*.

Results and discussion

Results. In this study, the possibility of using several morphological traits in identification of female castes of *B. ignitus* and *B. ardens* was evaluated. Even with statistically significant differences of some morphological characters between two species, overlapping quantitative traits hindered accurate identification of the species (Table 1, Figs. 3 & 4).
However, using 435 bp of COI gene and AluI, BspHI and EarI restriction enzymes allowed molecular identifications of these two species with unique profiles from the digestion by these restriction enzymes (Table 2, Fig. 5). We also demonstrated that this method can be applied to identification of older specimens with some morphological characters damaged.
Conclusion. The results demonstrated that reliable morphologically-based identification of females of these two species are not possible and the developed PCR-RFLP method can be used as an alternative option in identification.

Table 1. Morphological measurements for female castes of *Bombus ardens* and *B. ignitus*

Trait	*B. Ardens*		*B. ignitus*		P value
	Range	mean±SD	range	mean±SD	
HH / HW	0.94-1.3	1.08±0.06	0.89-1.027	0.97±0.03	<0.001
GH / GW	0.6-0.91	0.756±0.6	0.33-0.66	0.56±0.06	0.399
TIL / TIW	2.65-3.62	3.03±0.2	2.43-3.37	2.94±0.2	0.827
BAD / POD	0.41-0.6	0.52±0.04	0.43-0.64	0.53±0.05	0.485
SL / HW	0.37-0.51	0.42±0.03	0.42-0.56	0.48±0.03	0.751
POD / HW	0.22-0.28	0.25±0.01	0.22-0.28	0.25±0.01	0.576
LOD / POD	0.4-0.6	0.49±0.04	0.43-0.63	0.51±0.04	0.43
TL / TW	1.01-1.37	1.22±0.07	1.09-1.58	1.29±0.08	0.318
HH / GH	6-7.8	6.99±0.4	7.12-10.22	8.56±0.7	<0.001
WL / WW	3.04-3.9	3.43±0.18	2.9-4	3.45±0.23	0.123
ML / MW	1.95-2.73	2.32±0.17	1.8-2.66	2.25±0.15	0.362
SL / 2-4AL	1.66-2.2	1.9±0.1	1.6-2.7	2.02±0.16	<0.001

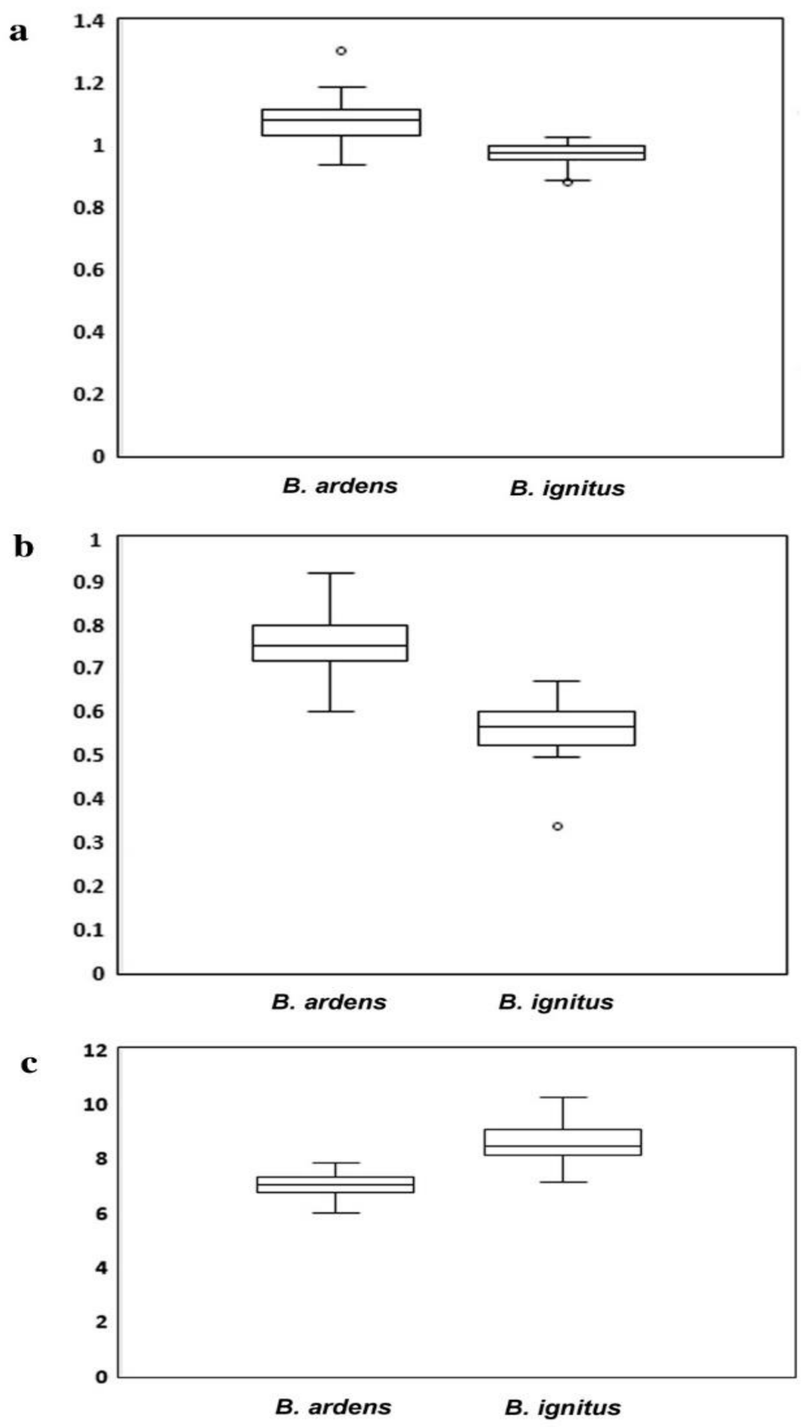
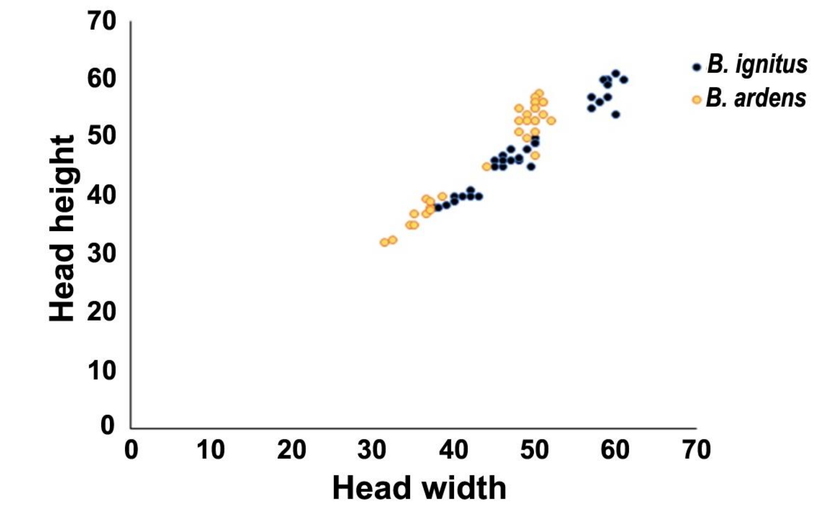


Figure 3. Relative measurements of HH to HW (a), GH to GW (b) and HH to GH (c) in *B. ardens* and *B. ignitus*



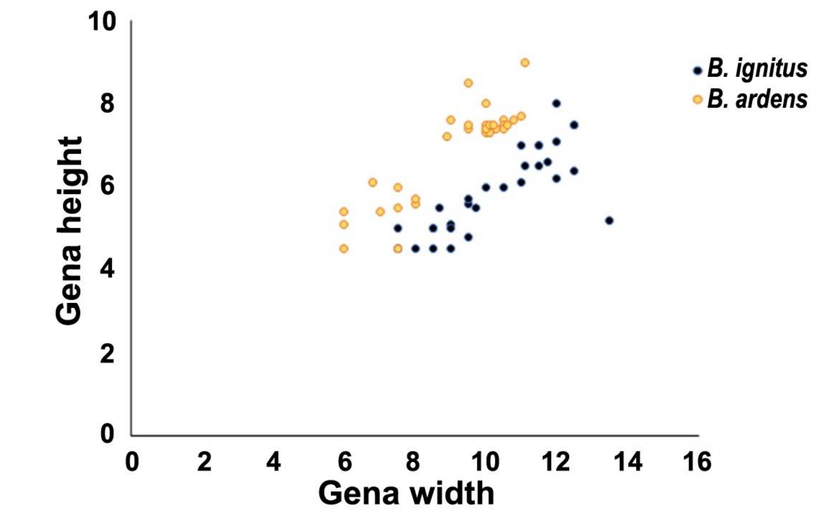


Figure 4. Combining the height and width of Gena (a) and height and width of head (b) in *B. ardens* and *B. ignitus*.

Table 2. The expected fragment lengths of the RFLP haplotypes of *Bombus ardens* and *B. ignitus* based on enzyme restriction sites of the 435-bp COI fragment

Species	Haplotypes	Restriction enzyme		
		AluI	BspHI	EarI
B. ardens	All haplotypes	435	435	435
B. ignitus	Haplotypes 1, 3-6, 11	15/336/84	291/144	209/226
	Haplotypes 2, 7-10	15/330/6/84	291/144	209/226

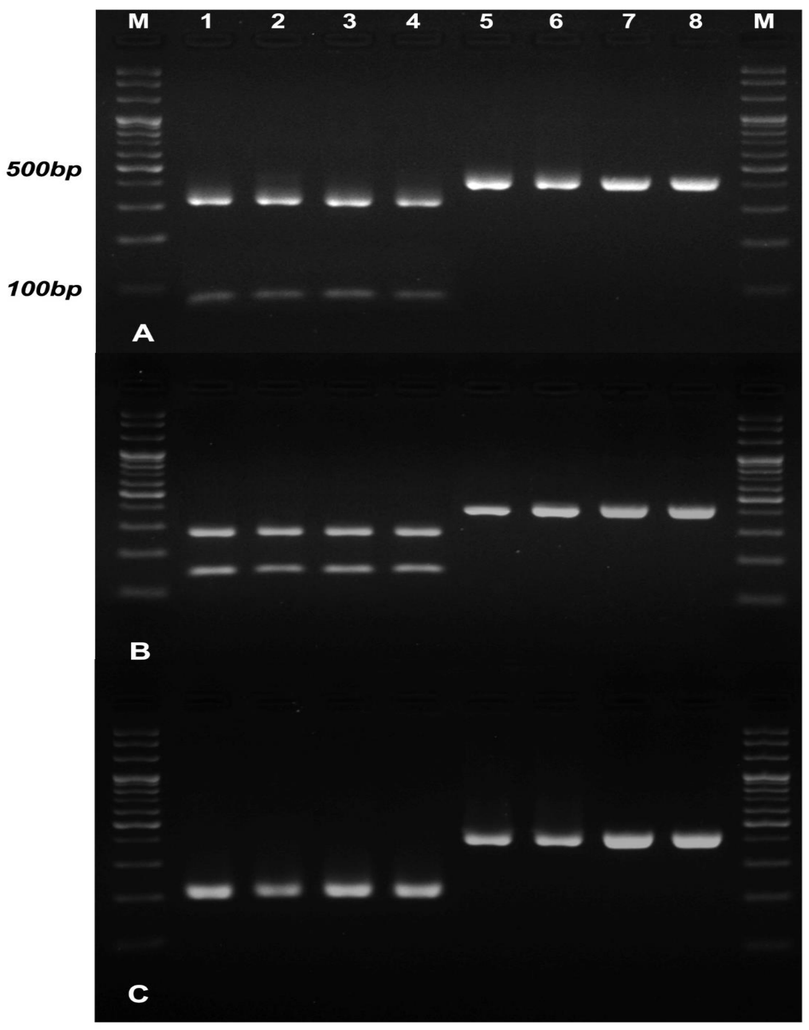


Figure 5. PCR-RFLP pattern for COI of Bombus spp. digested with AluI (A), BspHI (B) and EarI (C) restriction endonuclease. Lane 1-4 and 7, *B. ignitus*; lane 5-6 and 8; *B. ardens*; M, 100bp ladder.