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Seasonal morphological variation in a pollinator community: the wild bees of Southern Corsica

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I am quite convinced that Insects offer better or clearer illustrations of the problems you occupy yourself with than any other class of animals or plants. It is so easy with them to obtain great series of examples & have them before you in a small compass, which is one advantage they have.

Henry Walter Bates
Letter to Charles Darwin
28 March 1861

INDEX

Sommario

Le api selvatiche (Hymentoptera: Apoidea: Anthophyla) sono un gruppo monofiletico di circa 20’000 specie, che costituisce il più importante gruppo di insetti impollinatori in habitat temperati e tropicali in tutto il pianeta (Klein et al., 2007; Garibaldi et al., 2013; Ballantyne et al., 2017). Ogni comunità può comprendere anche 200 specie di api che, pur accomunate da una simile dieta basata su nettare o polline, presentano una grandissima variabilità nel comportamento, nella fenologia e nella forma corporea (Michener, 2007; Peters et al., 2016). La variabilità nella taglia corporea è particolarmente significativa, poiché la massa corporea può variare di 200 volte tra i due estremi dimensionali della comunità (Michener, 2007; Osorio-Canadas et al., 2016; Peters et al., 2016). Nel tentativo di individuare quali fattori ambientali influiscano maggiormente sulla variabilità morfologica delle comunità di api, molti autori si sono concentrati sul ruolo della temperatura ambientale.

Una legge fondamentale della biogeografia, la legge di Bergmann (Bergmann, 1847), descrive la variazione geografica della taglia corporea degli animali endotermi lungo un gradiente di temperatura: l’autore afferma che la taglia corporea dovrebbe essere positivamente correlata alla latitudine, poiché la temperatura diminuisce all’aumentare della latitudine (in valore assoluto). Una maggiore taglia corporea corrisponde geometricamente a un ridotto rapporto superficie/volume (S/V), e dunque si riduce la perdita di calore e il costo della termoregolazione (Bergmann, 1847; James, 1970; Watt et al., 2010; Shelomi, 2012).

Molte specie di api possiedono la capacità di regolare fisiologicamente la temperatura corporea, in particolare, per mantenere la muscolatura toracica entro un range di temperatura che garantisca un volo efficiente (Heinrich, 1993): sono, dunque, endoterme. Tale termoregolazione, effettuata tramite la contrazione della muscolatura indiretta (Esch e Bastian, 1968), ha un’efficienza variabile nelle api selvatiche, risultando assente o ridotta in alcune specie (generalmente le più piccole e solitarie) e più elevata in altre (in genere di grandi dimensioni e sociali) (Bishop e Armbruster, 1999). Questo meccanismo fisiologico può permettere il mantenimento di una temperatura corporea ben al di sopra della temperatura ambientale, ma è particolarmente costoso dal punto di vista energetico, e ci si attende che ogni carattere che possa ridurne il costo sia positivamente selezionato (Gérard et al., 2018). Uno di questi caratteri potrebbe essere un ridotto rapporto S/V (dovuto ad una maggiore taglia corporea), e dunque negli ultimi trent’anni molti autori si sono dedicati allo studio della legge di Bergmann nelle api.

Una relazione positiva tra taglia corporea e latitudine è stata osservata in molti gruppi di api selvatiche, anche se il fenomeno non è generale e sono noti casi di pattern opposti, ad esempio nel genere *Bombus* (Hawkins, 1995; Ramirez-Delgado et al., 2016; Gérard et al., 2018). La temperatura ambientale è influenzata

anche dall'altitudine e dalla stagionalità, e pattern di Bergmann altitudinali e stagionali sono stati osservati, in alcuni casi, in comunità di api (Osorio-Canadas et al., 2016; Peters et al., 2016; Classen et al., 2017).

Si ritiene che altri fattori ambientali possano influenzare l'evoluzione della taglia corporea negli animali, come l'abbondanza di risorse alimentari, con risposte sia adattative, sia plastiche (Boyce 1979; Geist, 1987; Geist, 1990; Atkinson and Sibly, 1997; Watt et al., 2010; Gérard et al., 2018). È stato ipotizzato che la taglia corporea possa essere positivamente correlata alla produttività primaria, e questo effetto è stato osservato in alcuni vertebrati (Rosenzweig, 1968; McNab, 1971; Yom-Tov and Nix, 1986; Geist, 1990). Sono state proposte delle spiegazioni adattative per questa correlazione (Boyce, 1979) ma non è ancora noto se un simile effetto statistico sia osservabile nelle api, né se sia osservabile su una scala diversa da quella latitudinale, ad esempio lungo un intervallo di tempo stagionale.

Questo lavoro si concentra sullo studio delle variazioni stagionali della taglia e di altri caratteri morfologici nella comunità di api selvatiche della Corsica Meridionale, cercando di indagare gli effetti di due fattori ambientali (temperatura e abbondanza di risorse alimentari) sulla variazione della taglia corporea e di altri caratteri morfologici nel corso della stagione. Tali effetti implicherebbero un'influenza delle variazioni stagionali di temperatura e abbondanza di risorse sull'evoluzione della taglia corporea e della fenologia nelle api (Osorio-Canadas et al., 2016). La taglia corporea, nelle api, è fortemente correlata alla lunghezza della lingua, come già noto (Cariveau et al., 2016), e dunque, a variazioni stagionali nella taglia dovrebbero corrispondere simili variazioni stagionali nelle dimensioni dell'apparato boccale, con possibili conseguenze sull'interazione tra le api e le piante a fiore di cui sono i pronubi (Harder, 1983; Borrell, 2005).

Lo scopo di questa tesi è, dunque, verificare la presenza di un pattern di Bergmann stagionale in una comunità di api, ovvero una taglia corporea media maggiore all'inizio e alla fine della stagione, e di verificare se la variazione stagionale è correlata alla variazione di temperatura ambientale. Inoltre, si vuole indagare la presenza di un effetto dell'abbondanza di risorse alimentari sulla taglia corporea.

Infine si vuole verificare se al pattern di variazione stagionale della taglia corrisponda un pattern di variazione stagionale nella lunghezza degli apparati boccali nella comunità.

Per rispondere a queste domande è stata utilizzata una collezione entomologica derivante da un monitoraggio faunistico effettuato da Marzo a Novembre 2017 nei dintorni di Bonifacio (Corsica, Francia). 391 femmine, appartenenti a 121 specie sono state sottoposte ad alcune misurazioni morfometriche, per ottenere, per ciascuna specie, i valori medi della massa corporea, della taglia corporea (utilizzando una proxy standard, la distanza intertegulare, ITD, ovvero la distanza tra le *tegulae*, i due scleriti toracici che proteggono l'articolazione delle ali anteriori) (Cane, 1987; Kendall et al., 2018), e del rapporto S/V (basato su una rudimentale descrizione della forma corporea). Per un campione ridotto (114 specie) è stata misurata la densità (lineare) delle *setae* toraciche. Un database di lunghezze degli apparati boccali (in particolare, la lingua, intesa come somma di premento e *glossae*) è stato utilizzato (i dati erano disponibili per 95 specie).

Per ottenere una stima della temperatura di volo degli individui e delle specie considerati (nell'ultimo caso, una temperatura di volo minima o media osservata per ciascuna specie) abbiamo utilizzato un database pubblico di dati meteorologici (ECAD 17.0) (Haylock et al., 2008).

La massa corporea è stata utilizzata per calcolare l'andamento della biomassa totale della comunità nel corso dell'anno. La biomassa totale è stata poi utilizzata come proxy dell'abbondanza delle risorse alimentari.

Abbiamo studiato l'andamento stagionale della taglia corporea e del rapporto S/V per verificare se la comunità fosse composta, in media, da api più grandi (con un ridotto rapporto S/V) agli estremi della stagione e più piccole in estate, come predetto dalla legge di Bergmann stagionale (Osorio-Canadas et al., 2016).

Successivamente, seguendo un approccio comparativo, abbiamo studiato la relazione tra la taglia corporea (e il rapporto S/V) e la temperatura ambientale media (o minima) osservata per ciascuna specie, anche tenendo conto della filogenesi, utilizzando un albero filogenetico dei generi di api selvatiche, opportunamente modificato per renderlo applicabile al nostro dataset (Hedtke et al., 2013).

Abbiamo studiato l'effetto della temperatura ambientale e dell'abbondanza di risorse sulla morfologia media della comunità (cioè, sulla media ponderata della taglia corporea o del rapporto S/V ad una data condizione ambientale) per verificare se questi due fattori avessero un effetto significativo sulla composizione della comunità, anche tenendo conto, per un sottoinsieme del nostro campione, del possibile effetto tampone determinato da una maggiore densità di *setae* sul torace (Peters et al., 2016). Infine, abbiamo studiato l'andamento stagionale della lunghezza media della lingua nella nostra comunità, per verificare se l'andamento di tale carattere fosse o meno simile all'andamento della taglia corporea.

I risultati che abbiamo ottenuto sono i seguenti:

- La comunità sembra essere mediamente composta da api più grandi all'inizio e alla fine della stagione. Delle semplici analisi di diversità mostrano che questo è in parte dovuto all'avvicendarsi, durante l'anno, di specie e gruppi filetici con diversa morfologia e fenologia.
- Dividendo le api presenti nella comunità in gruppi corrispondenti alle 5 famiglie presenti, si osserva che tre famiglie (Apidae, Colletidae, Megachilidae) mostrano un pattern di Bergmann stagionale, presentando

una maggiore abbondanza di api di dimensioni ridotte in estate e di dimensioni maggiori in primavera e autunno. Due famiglie (Andrenidae e Halictidae) mostrano un pattern opposto.

- L'approccio comparato classico mostra un effetto statisticamente significativo della temperatura solo nel caso della taglia corporea nei Colletidae.
- Il segnale filogenetico è abbastanza elevato per la taglia corporea, il rapporto S/V e la temperatura media specifica. L'approccio comparato filogenetico non mostra effetti significativi della temperatura né sulla taglia corporea né sul rapporto S/V.
- Si osserva un effetto statistico significativo della temperatura ambientale sulla media ponderata della taglia corporea e del rapporto S/V per tutti i gruppi (eccetto gli Andrenidae). L'effetto sulla taglia corporea è negativo per tre famiglie (Apidae, Colletidae e Megachilidae), mentre è positivo per gli Halictidae. Il rapporto S/V mostra un andamento opposto.
- Un effetto significativo della biomassa sulla taglia corporea è stato osservato per una famiglia (Halictidae) mentre è stato osservato per tre famiglie nel caso del rapporto S/V (Apidae, Colletidae e Halictidae). L'effetto è positivo nel primo caso, negativo nel secondo.
- Si osserva un effetto negativo della densità delle *setae* sul torace sul rapporto S/V per tre famiglie (Apidae, Colletidae, Megachilidae), contraddicendo dunque la previsione secondo cui una peluria più fitta dovrebbe essere correlata a rapporto S/V superiore alla media.
- La lunghezza della lingua è fortemente correlata alla taglia corporea. L'andamento stagionale della media della lunghezza della lingua nella comunità è simile a quello della taglia corporea, con lingue in media più lunghe all'inizio e alla fine della stagione, e lingue di lunghezza ridotta in estate. Analizzando l'andamento per le singole famiglie, per tutte le famiglie, con l'eccezione degli Andrenidae, si osserva un pattern molto simile a quello della taglia corporea.

Questo lavoro mostra, anzitutto, che in una comunità mediterranea di api selvatiche la distribuzione della taglia corporea cambia durante l'anno, e la comunità, pur presentando per tutto l'anno specie con taglie corporee che coprono buona parte del range dimensionale della comunità, è composta maggiormente di api appartenenti a specie di grandi dimensioni nei periodi più freddi, e, al contrario, di api di piccole dimensioni in estate. Questo andamento risulta valido per la comunità nel suo insieme e per tre famiglie delle cinque osservate nel campionamento. La presenza di un pattern così evidente giustifica la ricerca di meccanismi (adattativi o plastici) che possano spiegare una simile strutturazione.

L'approccio comparativo classico e quello filogenetico non mostrano particolari effetti significativi della temperatura media (o minima) di raccolta delle specie sulla taglia corporea (tranne nel caso dei Colletidae) o sul rapporto S/V. Poiché simili effetti sono stati osservati in uno studio precedente (Osorio-Canadas et al., 2016), si può forse supporre che il segnale portato dalle stime delle temperature di volo specifiche da noi utilizzate sia ridotto o che la filogenesi da noi utilizzata non sia abbastanza accurata.

Risulta invece particolarmente interessante il risultato delle analisi dell'andamento della taglia corporea media (e del rapporto S/V medio) della comunità in funzione di due fattori ambientali: la temperatura di volo individuale e la quantità di risorse disponibili (sostituita nelle nostre analisi dalla biomassa totale della comunità nel mese di raccolta dell'individuo). Queste analisi, che, essendo effettuate sul dataset complessivo della collezione, dipendono anche dalle abbondanze specifiche, mostrano un effetto statistico significativo delle variabili considerate su alcuni gruppi filetici. Osserviamo un effetto negativo della temperatura sulla taglia corporea (positivo su S/V) per Apidae, Colletidae e Megachilidae, e dunque queste tre famiglie si conformano alle previsioni basate sull'ipotesi di Bergmann: infatti, in queste famiglie, si osserva che le api di grandi dimensioni (con ridotto rapporto S/V) tendono ad essere più abbondanti (sul totale della comunità) in condizioni di bassa temperatura ambientale. Una famiglia (Halictidae) mostra un pattern opposto.

Questo risultato suggerisce che la selezione per un ridotto sforzo di termoregolazione potrebbe aver influito sull'evoluzione della taglia corporea nelle api, e sulla distribuzione delle varie specie nella stagione. Ciò nonostante, questa ipotesi richiede ulteriori approfondimenti, includendo possibilmente dati sulle reali capacità di termoregolazione delle varie specie (Bishop e Armbruster, 1999) e sulla variabilità morfologica intraspecifica (Shelomi, 2012).

Osserviamo un effetto negativo della densità di *setae* toraciche sul rapporto S/V per tre famiglie (Apidae, Colletidae e Megachilidae, e ciò contraddice la nostra previsione secondo cui una peluria più densa dovrebbe agire da tampone sul costo della termoregolazione e dunque consentire un rapporto S/V maggiore, in presenza della stessa temperatura ambientale, rispetto ad api con una minore densità di *setae*. È possibile che tale risultato sia dovuto al fatto che il nostro modello considera solamente la densità delle *setae* e non la loro lunghezza, quando la lunghezza delle *setae* toraciche sembra essere correlata negativamente alla temperatura minima di volo nelle api (Peters et al., 2016).

Allo stesso tempo si osserva un effetto significativo dell'abbondanza di risorse sulla taglia corporea (positivo, solo negli Halictidae) e sul rapporto S/V (negativo, per Apidae, Colletidae e Halictidae). Questo significa che da una a tre famiglie (a seconda del metodo utilizzato) presentano una taglia corporea mediamente maggiore in periodi di maggiore abbondanza di risorse (questo perché taglia corporea e S/V sono negativamente correlati). Un simile effetto sulla distribuzione della taglia corporea in comunità di api nel corso della stagione non è mai stato osservato, e non sono stati proposti meccanismi esplicativi. Una correlazione tra l'abbondanza di risorse alimentari (o la produttività primaria) e la taglia corporea è stata osservata lungo gradienti latitudinali in alcuni vertebrati, ma anche in questo caso i meccanismi sono discussi (Watt et al., 2010). Questo lavoro mostra che è possibile osservare un effetto dell'abbondanza di risorse alimentari sulla taglia corporea su scala stagionale, anche separando tale effetto da quello della temperatura ambientale, nonostante questi due fattori siano parzialmente correlati (Yom-Tov and Nix, 1986). Sarebbe necessario approfondire lo studio di questa correlazione, almeno introducendo misure dirette e ad una scala temporale più fine delle risorse alimentari usate dalle api.

In generale, osserviamo una correlazione tra due parametri ambientali e la distribuzione della taglia corporea nella comunità nel corso della stagione. Questo può significare che entrambi i fattori potrebbero aver influenzato l'evoluzione della taglia corporea e della fenologia nelle api selvatiche.

Infine, i nostri dati confermano la forte correlazione tra la taglia corporea e la lunghezza della lingua nelle api (Cariveau et al., 2016). Inoltre, la distribuzione della lunghezza della lingua nella comunità varia durante l'anno in modo molto simile alla quella della taglia corporea, probabilmente a causa della correlazione tra i due caratteri.

La lunghezza della lingua può influenzare molti aspetti della relazione tra le api e le piante di cui sono i pronubi (Plowright and Plowright, 1999; Borrell, 2005). Dunque, questo lavoro suggerisce che la variazione stagionale della temperatura ambientale e della disponibilità alimentare potrebbe aver influito sull'evoluzione della taglia corporea nelle api, a sua volta modificando indirettamente il processo coevolutivo che lega le api alle piante a fiore, con conseguenze ancora non ben comprese per entrambi i gruppi.

1. INTRODUCTION

Wild bees are a highly specious group of Hymenoptera, showing an important range of morphometric variation, especially in size and shape (Michener, 2007). The origin of this variation has long been investigated, and some groups of wild bees proved to follow Bergmann's rule, a fundamental rule in biogeography (Hawkins, 1995; Shelomi, 2012; Gérard et al., 2018).

Proposed at first by Bergmann to describe the relationship between body size and latitude in endotherm animals (Bergmann, 1847), this rule has been widely investigated in several taxonomic groups, both endotherms and ectotherms (Shelomi, 2012). The main explanation, already proposed by Bergmann, is that environmental temperature and thermoregulation ability play a major role in the evolution of body size and shape in animals (Bergmann, 1847; Watt et al., 2010): recent research tried to extend this rule to other temperature gradients, such as altitudinal and seasonal gradients (Osorio-Canadas et al., 2016; Peters et al., 2016; Classen et al., 2017). Though Bergmann's rule seems not to be a general rule for bees, some groups show size patterns congruent with Bergmann's rule along various temperature gradients, including the seasonal gradient (Osorio-Canadas et al., 2016; Peters et al., 2016; Gérard et al., 2018).

In this thesis, we studied the seasonal variation of body size and other morphological traits in a Mediterranean wild bee community: we investigated the presence of a Bergmann's seasonal rule in the community, including consideration about body shape; moreover, we looked for an effect of the seasonal variation of the abundance of food resources on body size. This allowed us to investigate the possible effects of two environmental factors on body size, body shape and phenology evolution in wild bees. Finally, we analysed the seasonal variation of the length of the tongue in the community, to identify the relationship with the variation in body size. This relationship could affect the plant-pollinator relationship that ties bees to flower plants.

1.1. *Sensu lato* Bergmann's rule

Evolutionary biologists and zoologists have long been interested in body shape and size evolution among animal taxa, as these traits can show important intra- and interspecific variation and can be related to individuals' fitness (Adams et al., 2004; Adams et al., 2013). In the last decades, quantitative measurements of morphological traits have been related to environmental condition (such as temperature or predation rate) (*e.g.* Trussell and Smith, 2000; Kitano et al., 2008; Peters et al., 2016), behavioural traits (such as social structure, mating system type or level of sexual conflict and promiscuity) (*e.g.* Plard et al., 2011; Dines et al., 2014), and, in general, life history traits (such as lifespan or reproductive effort) (*e.g.* Garcia-Barros, 2000), both at an intra- and an interspecific level.

In this context, the effect of spatial or temporal variation of environmental temperature on body size and shape evolution has been deeply investigated in the last 150 years (Shelomi, 2012). At first, the rule proposed by Bergmann predicted a positive relationship between body size and latitude among endothermic animals (Bergmann, 1847; Watt et al., 2010; Shelomi, 2012). Bergmann described a pattern and proposed an explanatory mechanism (James, 1970; Geist, 1990; Watt et al., 2010), so that Geist recognised two different components of Bergmann's rule (Geist, 1990; Watt et al., 2010). The first is the pattern: "body size varies inversely with ambient temperature, so that body size increases with latitude" (Bergmann, 1847; Geist, 1990; Watt et al., 2010). The second is the explanatory mechanism: ''large size is adaptive in cold environments, because the surface area decreases relative to mass as the 2/3 power of mass'' (Bergmann, 1847; Geist, 1990; Watt et al., 2010). Therefore ''large animals lose less heat relative to mass, and large size in cold environments is an adaptation for energy conservation" (Bergmann, 1847; Geist, 1990; Watt et al., 2010). Bergmann tested the rule at an interspecific level in a group of domestic animals, with poor results (Bergmann, 1847; Watt et al., 2010), but since then, many works raised evidence that Bergmann's clines (*i.e.* positive correlation between latitude and body size) are present in many groups of animals (Watt et al., 2010 for a brief review).

The exact definition and the range of application of Bergmann's rule have long been debated (Watt et al., 2010). Some proposed that Bergmann's and other similar rules are only empirical rules, valid at an intraspecific level (Mayr, 1956; Mayr, 1963; James, 1970) while others extend it to the interspecific level (Bergmann, 1847; Shelomi, 2012). Bergmann at first referred to different species within genera (Bergmann, 1847; Watt et al., 2010) and the intraspecific interpretation belongs to different authors, such as Rensch and James (in fact, intraspecific Bergmann's rule is sometimes called James's rule) (Rensch, 1938; James, 1970; Watt et al., 2010; O'Gorman et al., 2012). The first definition of Bergmann's rule was long misunderstood, as the original work was written in German, and further interpretation usually came from a small number of incomplete translations, mostly from a 1938 interpretation by Rensch (Rensch, 1938; Watt et al., 2010; Shelomi, 2012). Nevertheless, this rule is considered fundamental in biogeography, and has been widely investigated and tested among endotherms, from the intraspecific to the community level (James, 1970; Ashton et al., 2000; Ashton, 2001; Ashton and Feldman, 2003; Blackburn et al., 1999; Millien et al., 2006; O'Gorman et al., 2012; Shelomi, 2012; Gérard et al., 2018).

Bergmann's rule was originally proposed for endothermic vertebrates, but similar body size patterns are observed and studied in other groups: ectothermic vertebrates, endothermic and ectothermic invertebrates (Angilletta and Dunham, 2003; Watt et al., 2010; Shelomi, 2012). The extension of the rule to ectothermic taxa has been widely criticized, as ectotherms, by definition, do not share the same physiological thermoregulatory mechanisms with endotherms (Shelomi, 2012). In fact, when referring to Bergmann's rule in these cases, authors only refer to the ecological pattern, and not to the underlying mechanism (Watt et al., 2010). It has also been argued that, in this case, a rule describing similar patterns that are likely to be caused by different mechanisms can't be a valid scientific law (Watt et al., 2010). Nonetheless, these studies have been widely conducted and show interesting results, so that the name "Bergmann's rule" is widely accept when referring to patterns with higher body size in colder climates (Meiri, 2011; Gérard et al., 2018). Some refer to any extension of the original definition as to *sensu lato* Bergmann's rule (Shelomi, 2012).

Besides its relationship with latitude, temperature varies also along altitude, season and other geographic or temporal gradients, and altitudinal and seasonal Bergmann's clines were observed at a community level (Osorio-Canadas et al., 2016; Peters et al., 2016; Classen et al., 2017). These results respect the prediction that, if selection on thermoregulation is central in shaping body size evolution in animals, these clines should be observed (Osorio-Canadas et al., 2016). Moreover, in the case of the validity of a seasonal Bergmann's rule, it would be possible to infer that thermoregulation and body size play a role in structuring community phenology (Osorio-Canadas et al., 2016).

Body size evolution is certainly influenced by other factors than thermoregulation, for instance, locomotion abilities (*e.g.* flight performances, and hence predation risk, foraging abilities, etc.) or resource allocation (Osorio-Canadas et al., 2016). Thermoregulation itself can be influenced by other factors than surface-to-volume ratio: behaviour (Willmer and Stone, 2004), physiology (Heinrich, 1993; Peat et al., 2005a), morphological traits such as fur or hair density and length (Heinrich, 1993; Peters et al., 2016), or appendices length and shape (Peters et al., 2016). Moreover, some proposed that even environmental temperature can have other effects than influencing thermoregulation efforts, by acting on growth rate during the development, which could be a possible explanation of Bergmann's clines in ectotherms (Van Voorhies, 1996; Atkinson and Sibly, 1997; Angilletta and Dunham; 2003; Angilletta et al., 2003).

Some proposed, both for endotherms and ectotherms, that phenotypic plasticity and resource or food availability can play an important role in shaping size patterns (Atkinson, 1994; Atkinson and Sibly, 1997; Watt et al., 2010). It has been argued that body size should be positively influenced by resource availability, and hence, that all the rest being equal, a higher amount of available resources (*i.e.* more food or less competition for it) should lead to bigger sizes, both with adaptive and plastic responses (Boyce, 1979; Geist, 1987; Geist, 1990; Atkinson and Sibly, 1997; Watt et al., 2010). It has also been proposed that size is strictly correlated to primary productivity, and similar clines between size and productivity were observed in mammals (Rosenzweig, 1968; Yom-Tov and Nix, 1986; Geist, 1990). Some noticed that, "because biomass productivity and ambient temperature are related, it is difficult to separate the effects" (Yom-Tov and Nix, 1986; Watt et al., 2010). Boyce also proposed an adaptive explanation to describe these patterns along latitudinal gradients, that is called seasonality hypothesis (or fasting endurance hypothesis, or starvation hypothesis) (Boyce, 1979; Watt et al., 2010): it states that large body size facing higher resource availability is favoured in habitat with unpredictable seasonal resources, because larger animals can grow and accumulate resources at a faster rate than the small ones, so that their survival rate is higher (Boyce, 1979; Watt et al., 2010). For these reasons, many suggested that patterns in body size have multiple causes and are the result of complex trade-offs (Angilletta and Dunham, 2003; Osorio-Canadas et al., 2016; Gérard et al., 2018).

1.2. Wild bees as a model

Wild bees (Hymenoptera: Apoidea: Anthophila) have been the object of investigation about *sensu lato* Bergmann's rule validity in an invertebrate taxon (Shelomi, 2012; Gérard et al., 2018). This group is highly diverse, counting more than 20 000 species in tropical and temperate climates worldwide (Michener, 2007; Gérard et al., 2018). A wild bee community can include more than 200 species, with up to a 200-fold interspecific (and relatively low intraspecific) variation in size (Michener, 2007; Osorio-Canadas et al., 2016; Peters et al., 2016; Gérard et al., 2018). They are considered the most important pollinators in natural and agricultural ecosystems (Klein et al., 2007; Garibaldi et al., 2013; Peters et al., 2016; Ballantyne et al., 2017) and are sensitive to climate change, with marked declines in many groups (Biesmeijer et al., 2006; Williams and Osborne, 2009; Bommarco et al, 2011; Osorio-Canadas et al., 2016; Peters et al., 2016). These characteristics make them interesting to study both as a useful model for fundamental evolutionary biology (as insects often are, since the beginning of the discipline) (Bates, 1861), and as an important group of ecologically, socially and economically relevant species that present important conservation issues facing climate change (Osorio-Canadas et al., 2016; Peters et al., 2016).

Many bee species proved to be able to regulate body temperature with physiological mechanisms, and are, hence, endothermic species (Heinrich, 1980; Stone and Willmer, 1989; Heinrich, 1993; Stone, 1993; Stone, 1994; Peters et al., 2016). Thermoregulation, especially of thoracic temperature, is not rare in large insects (>50 mg of dry weight) (Heinrich, 1993) and is considered an adaptation to guarantee efficient mechanical power production during flight (Heinrich, 1993; Woods et al., 2005). Most studies about thermoregulation in bees were conducted on *Apis mellifera*, but similar mechanisms have been identified in other bee species (*e.g.* in *Amegilla*, *Anthophora*, *Bombus*, *Osmia*, *Xylocopa* and other genera) (Stone and Willmer, 1989; Heinrich, 1993; Stone, 1993; Stone, 1994; Vicens and Bosch, 2000). Endogenous heat production (also called thermogenesis) is thought to be a by-product of the activity of thoracic flight muscles, in particular, indirect flight muscles (Esch and Bastian, 1968; Heinrich, 1993; Harrison et al., 1996; Woods et al., 2005; Goulson, 2010; Peters et al., 2016). In general, before flying, bees must warm-up from a poikilothermic state, and then they must maintain a relatively high thoracic temperature during flight (*e.g.* over 40 °C in some *Anthophora* species) (Stone, 1994).

Since the process is extremely costly, endothermic bees are able to maintain a physiological flight temperature totally independent from air temperature only in a certain range of environmental temperature (*e.g.* 19-37 °C for *A. mellifera*) and they are not able to fly below a certain threshold (Heinrich, 1993; Woods et al., 2005; Peters et al., 2016). Different species show different thermoregulatory efficiency, with bigger or social bees, especially bumblebees, showing better thermoregulatory abilities than small solitary bees (Bishop and Armbruster, 1999). In general, the smallest species are not endothermic: in fact, a study conducted on 18 Alaskan wild bee species showed that there was a body mass threshold, below which bee species had no efficient thermoregulatory activity and were, hence, thermal conformers (Bishop and Armbruster, 1999). Another study found some indirect evidence of the presence of such threshold in a Spanish wild bee community (Osorio-Canadas et al., 2016). When referring to endothermic species, any trait that reduces the demand of energy for thermoregulation is expected to be favoured: this could reduce the warm-up time, increase the foraging efficiency and extend the flight period or the natural range of the species (*e.g.* to habitats with colder climates) (Osorio-Canadas et al., 2016; Peters et al., 2016; Classen et al., 2017).

One of these traits could be body size (and surface-to-volume ratio), and hence bees are expected to follow Bergmann's rule: in the last three decades many authors worked on this topic, often concentrating on the interspecific level (Hawkins, 1995; Peat et al., 2005a; Shelomi, 2012; Ramirez-Delgado et al., 2016; Scriven et al., 2016; Gérard et al., 2018). There is no consensus on how body size should be evaluated at a community scale: many authors worked on the distribution of species body size inside a genus or other taxonomic group (*e.g.* Hawkins, 1995; Ramirez-Delgado et al., 2016; Scriven et al., 2016), or on the mean of body size of the species inside a genus (*e.g.* Gérard et al., 2018) or in a community (*e.g.* Osorio-Canadas et al., 2016; Gérard et al., 2018). Many studies, usually conducted in temperate communities, found Bergmann's clines in different groups of wild bees, with some groups showing converse clines or no significant cline (Hawkins, 1995; Peat et al., 2005a; Shelomi, 2012; Ramirez-Delgado et al., 2016; Scriven et al., 2016; Gérard et al., 2018). A recent study, the first conducted on a continental scale, found overall Bergmann's clines in European wild bees, independently of social behaviour (sociality, solitary life and parasitism) or nesting behaviour (ground-nesting or stem-nesting bees) (Gérard et al., 2018). It also highlighted some genera with converse clines: *Bombus*, *Ceratina*, *Colletes* and *Melitta* (Gérard et al., 2018). The case of *Bombus* was already known (Peat et al., 2005a; Ramirez-Delgado et al., 2016) but debated (Scriven et al., 2016). Bergmann's extended rules were also tested in bee communities with positive results both for altitude (Peters et al., 2016; Classen et al., 2017) and season (Osorio-Canadas et al., 2016).

These studies on wild bees usually involve measurements of body length (Hawkins, 1995) or intertegular distance (ITD, also intertegular span, ITS) (*e.g.* Osorio-Canadas et al., 2016; Gérard et al., 2018) as proxies of body size. Only rarely is body mass used (*e.g.* Osorio-Canadas et al., 2016). The ITD is the distance between the two sclerites, called *tegulae*, that cover the articulation of forewings, and is strongly correlated with bee body mass (Cane, 1987; Gérard et al., 2018; Kendall et al., 2018). This way, most studies take into account body size, but they ignore information about body shape, making it hard to test mechanistic hypotheses: bees of the same size or mass with different shape could have, for example, important differences in their surface-to-volume ratio. Since insects in general do not show an overall validity of *sensu lato* Bergmann's rules, some argued that research in this field should focus on measuring multiple morphometric characters and testing mechanistic hypotheses (Shelomi, 2012).

In addition, other morphological traits (appendices length, colouration, hair length and density) (Peters et al., 2016) could influence thermoregulation in bees: for instance, hairless mutant honeybees showed higher basal metabolism than wild type bees (Southwick, 1985). Moreover, a study on altitudinal Bergmann's rule showed that thoracic hair length had a negative relationship with the thermal minimum (positive relationship with the altitudinal peak) of activity in many bee families (Peters et al., 2016).

Correlation between body size and primary productivity was observed in many animal taxa (Watt et al., 2010). By now, we do not have much information on this correlation among bees, even if a positive relationship between adult body size and food income was observed in a bee species (*Ceratina calcarata*) (Johnson, 1988; Johnson, 1990). Some authors suggest that food availability is among the major drivers of body size clines in bees (Gérard et al., 2018).

Investigating the patterns of body size in bee communities and their origin could shed some light on other ecological patterns in which bees are involved (Osorio-Canadas et al., 2016). Bee body size is positively related to proboscis length, *i.e.* the length of the tongue, and the trend is allometric (Cariveau et al., 2016). Proboscis length is central in shaping bee interaction with plants (Cariveau et al., 2016): it influences flower choice and specialisation (Peat et al., 2005b), foraging time and efficiency (Harder, 1983; Plowright and Plowright, 1997), and pollination success for host plants (Shimizu et al., 2014), shaping plant-pollinator coevolutionary patterns and both bee and plant speciation (Borrell, 2005; Shimizu et al., 2014). Body size itself has effects on these interaction, *e.g.* influencing pollination success (Vivarelli et al., 2011). For these direct and indirect effects of body size on pollination and foraging, the interest of understanding body size patterns in bees goes beyond the interest of understanding this complex evolutionary pattern (Osorio-Canadas et al., 2016).

1.3. Aim and context of the study

The aim of this study is to investigate the relationship b\etween seasonal variation of temperature and resource availability on phenology, body size and shape in a temperate wild bee community, and to test for Bergmann's mechanistic hypotheses about surface-to-volume ratio. The final aim is to shed some light on the effect of temperature and resource abundance seasonal variation on the structure of the community. To our knowledge, a similar study has only been done once, on a Mediterranean bee community, but the approach was focused only on Bergmann's rule, with ITD as a proxy of body size (Osorio-Canadas et al., 2016). Our study tries to extend the descriptive approach to a direct test of mechanisms.

Moreover, we studied the relationship between thoracic hair density and body size. Since thoracic hair length influences bee thermoregulation abilities (Peters et al., 2016), we expect that this is true also for hair density, and we provide an analysis of the effect of hair density on surface-to-volume ratio.

Finally, we verified if the positive relationship between body size and proboscis length (Cariveau et al., 2016) is valid for our community, and studied the seasonal variation of proboscis length in the community, to understand whether a variation in body size and shape could have indirect effects on the interaction between bees and the flower plant community.

We focused on a Mediterranean area for which data about the bee community were available for an entire year: Southern Corsica. In a small area around the city of Bonifacio, a late 19th century French entomologist, Charles Ferton, realised a collection of about 8,000 insects, mostly hymenopterans, now held at the *Muséum National d'Histoire Nature*lle in Paris (MNHN). Though being long understudied, it represents one of the few cases of a hymenopteran community for which a considerable amount of data is available on the status of bee biodiversity for more than a century. For this reason, a biodiversity survey of the bee community was performed in 2017 in the same area and sites (A. Cornuel-Willermoz, unpublished data), as part of a project on bee biodiversity financed by the Labex BCDIV. This way, this community can be studied for both fundamental ecology or evolution studies and climate change-related studies: our study is performed on the collection obtained during the 2017 survey.

Contrary to previous studies, we chose to perform several morphometric measurements on a whole bee community. This approach allowed us to take into account not only body size, but also body shape, the latter being previously overlooked in studies about Bergmann's rule in invertebrates.

Our specific questions are:

1. Do we observe a seasonal Bergmann's cline in our bee community, *i.e.* do we observe higher body size and lower surface-to-volume ratio in colder months?
2. Is the relationship between body size (or surface-to-volume ratio) and temperature consistent with Bergmann's mechanistic hypothesis, *i.e.* do we observe bigger sizes and lower surface-to-volume ratios at lower

environmental temperatures?
3. Is there an effect of resource availability?
4. Do we observe an effect of thoracic hair density on surface-to-volume ratio?
5. Do we observe a positive relationship between body size and proboscis length?
6. Do we observe changes in proboscis length in the community along a year?

2. MATERIALS AND METHODS

This work is based on an entomological survey performed in 2017, in the context of a wild bee and wasp biodiversity monitoring in Corsica, financed by the Labex BCDIV (https://labex-bcdiv.mnhn.fr/) and performed by the *Centre d'Ecologie et des Sciences de la Conservation* (CESCO, Paris) and the *Observatoire et Conservatoire des Insectes de Corse* (OCIC, Corte, France). The collection is held at MNHN. The author did not participate to the sampling.

Body mass measurements were performed at the Institute of Ecology and environmental Sciences (iEES) in Paris. Morphological measurements were performed at the *Plateforme de Morphométrie* (morphometrics laboratory) at MNHN in Paris.

2.1. Study sites

We studied the wild bee community of the southern end of Corsica, in the city of Bonifacio and its surroundings (South Corsica, France). This region has a typical Mediterranean climate, therefore, a temperate climate with dry hot summers and mild rainy winters. The area has one of the rare calcareous substrates of Corsica: this geological peculiarity is reflected by a peculiar plant community, with a high species richness, especially for flower plants, including many protected species (Bournerias et al., 1990). Hence, various patches of the territory of the municipality of Bonifacio are subject to some level of protection, with both sites of community importance (SCI, as defined by the European Commission Habitat Directive – 92/43/EEC) and other forms of protection (*e.g. Conservatoire du littoral* sites: French national seaside and lakeside conservation) (protectedplanet.net). Some terrestrial, freshwater and marine ecosystems are protected (see figure 2.1), with a maximum IUCN management category of IV (protectedplanet.net). The remaining land is covered by urban and agricultural areas.

Collections were made at 7 different study sites covering an area of about 50 km^2, and different environment, from the city centre to protected area, in a period of 9 months, from March to November 2017. Each study site includes 2 or 3 stations (see table 2.1 for a list of sites and stations, with GPS position and a brief description; geographical distribution is shown in figure 2.2). The choice of the sites was driven by two main factors: the will of covering areas explored by

Ferton's late 19th century collection, and the necessity to cover various environments, from urban, to agricultural, to protected areas.

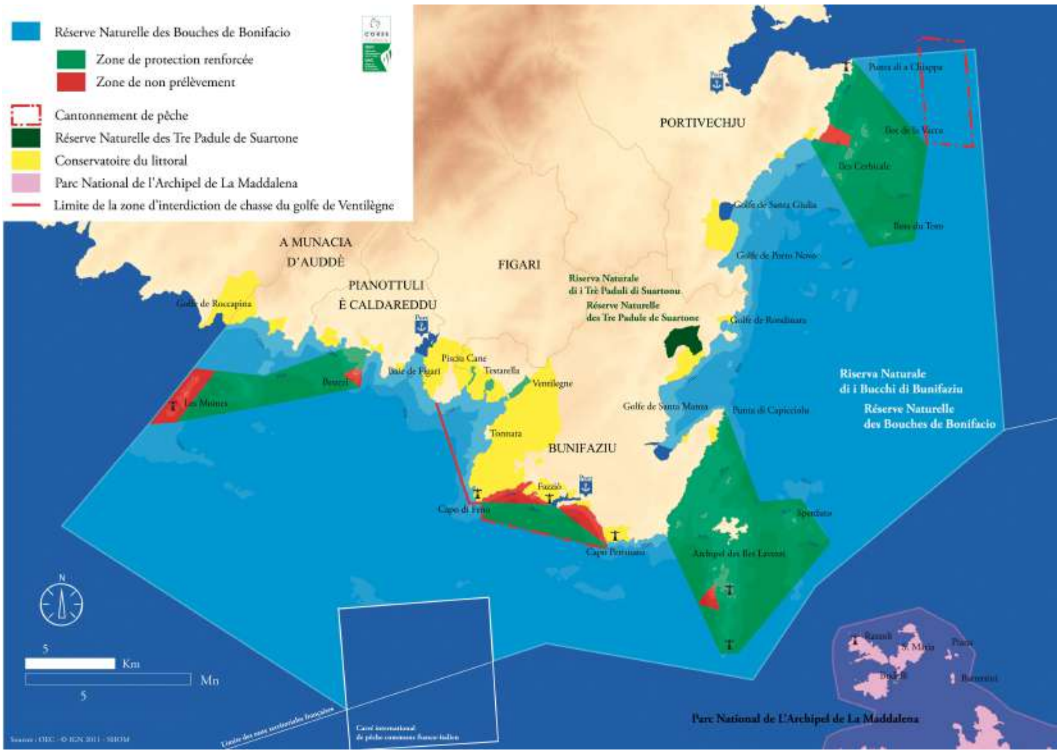

Figure 2.1: protected areas in the region of Bonifacio (SCIs are not displayed) (from rnbb.fr)

Figure 2.2: geographic location of study sites in the municipality of Bonifacio (red area, in yellow on the map of Corsica, topleft) (graphics A. Cornuel-Willermoz, with GoogleEarthPro)

Table 2.1: list of collection sites and stations for each site, with WGS 84 coordinates and description (A. Cornuel-Willermoz, unpublished data)

Station	Site name	Latitude	Longitude	Description
1A	Santa Manza	41.414531	9.237372	Dense maquis
1B		41.414325	9.237643	Maquis
2A	Route de Santa Manza	41.400568	9.216798	Xerophilous grassland and maquis
2B		41.400355	9.214525	Grassland and hedgerows
3A	Saint Julien	41.390343	9.180577	Fallow
3B		41.389786	9.179714	Fallow
4A	Bocca di Valle	41.378785	9.178601	Low shrubland and xerophylous grassland
4C		41.378538	9.178807	Low maquis
5A	Pertusato	41.368864	9.181344	Shrubland
5B		41.370934	9.181357	Low maquis
5C		41.371996	9.181751	Dense maquis
6A	Bonifacio centre	41.387842	9.157838	Shrubland
6B		41.386439	9.155151	Shrubland
7A	Mont de la Trinité	41.403898	9.120824	Dense maquis and rocks
7B		41.403416	9.120351	Shrubland

2.2. Collection protocol

Bees were sampled with two methods: sweep netting during transect walks and trapping with colour traps. The combination of these two methods is considered the most efficient choice in sampling wild bee communities, because they lead to complementary results: small-sized species are easily caught in traps and big-sized species usually occur more frequently in netting (Westphal et al., 2008; Wilson et al., 2008; Genoud and Langlois, 2013; Coiffait-Gombault et al., 2016).

Two transects were chosen for each study sites (14 in total) and 8 transect walks were performed on each of these transects every month, from March to November: for each transect, two repetitions of the walk were performed by two different collectors at two different times (morning, afternoon). Each transect walk consisted in catching every bee along a 25 metres-long and 4 metres-wide transect.

Pan traps of 3 different colours (white, blue and yellow) were left on the field for 48 hours in fifteen different stations in the 7 sites, twice a month, from March to November. The chosen colours and timings are considered the best choice for this kind of sampling (Westphal et al., 2008; Wilson et al., 2008).

Bees caught in transects were killed with ethyl acetate. Each bee was mounted on a pin and identified to the species level by experts in the different groups. The pins used to mount the insects were mostly size 1 pins from the Kabourek brand, but some individuals were mounted with minutiae (small size pins) from Kabourek or pins from other brands (Karlsbader, Austerlitz or Entomo Sphinx). Several bees were collected, either by trapping or random collection, in the same period and sites without following the protocol (A. Perrard, personal communication). These bees were not used to estimate species abundances, but some of them were used for morphometric analysis, to increase the available sample size for some species. We assumed that there was no within-species shape- or size-related collection bias among the three different methods (netting, trapping, random collection).

2.3. Study sample

The identification showed a sampled biodiversity of 152 bee species from a total of 3444 individuals. Collections made outside protocols provided other 1,400 individuals (for a total of about 170 species, considering specimens both in and outside protocol). Only females were collected for 57 species, and only males for 21 species.

Several bee species show important intersexual variation in size and shape (Michener, 2007), so we decided to perform morphometric analyses only on one sex, and we chose females to maximise the number of studied species. Each species with at least one intact female collected inside the protocol was selected, and 1 to 5 intact individuals (3.2 on average) of the species were chosen for morphometric analysis, obtaining a study sample of 121 species and 391 individuals (see table A1 in the appendices).

Only worker bees were used for eusocial species (*e.g. Bombus* genus). When possible, individuals were chosen to cover the entire observed flight period of the species, in order to reduce any effect of intraspecific variation (*e.g.* due to phenotypic plasticity) on size and shape on our analyses. We therefore assumed that variation in size and shape at an intraspecific level should be smaller than the interspecific variation. This assumption has already been made in previous literature (Osorio-Canadas et al., 2016; Peters et al., 2016), and is consistent with our preliminary analysis (see 3.3). Moreover, given the small number of collected individuals for many species, a community-wide study including evaluation of intraspecific variation would have been impossible.

2.4. Life history and behavioural traits of the studied species

The sample is composed of 5 different bee families: Andrenidae, Apidae, Colletidae, Halictidae and Megachilidae.

Bee species can differ in many different life history and behavioural traits (Wilson, 1971; Michener, 2007). Sociality, nesting behaviour, lecty and voltinism are usually considered among the most important in shaping bee ecology (Wilson, 1971; Willmer and Stone, 2004; Michener, 2007; Gérard et al., 2018). Information on at least one of these four traits were available for 114 of the 121 species of our sample (S. Roberts, unpublished data).

The complexity of social interaction within bee species can vary a lot, even in small groups of closely related species (e.g. tribes, genera, subgenera), from the solitary to the eusocial, to the kleptoparasite (Wilson, 1971). In solitary bee species, bees lay their eggs in a nest, provide the larvae with floral resources (mainly pollen) and leave the offspring (Michener, 2007). Social species are usually subdivided into 5 different categories, with a growing level of parental care and social interaction: subsocial, communal, quasisocial, semisocial and eusocial (Wilson, 1971). The latter presents cooperative brood care, separation in castes and overlapping generations (Wilson, 1971). Semisocial bees are similar to eusocial, without overlapping generations (Wilson, 1971). Kleptoparasitic bees lay their eggs inside the nests of other species, and the larvae feed on the floral resources provided by the host species (Michener, 2007).

Among the 99 species for which we have data about the social behaviour, 77 species (77.8%) are solitary, 1 is semisocial (1%), 16 species are eusocial (16.2%) and 8 are kleptoparasitic (8%). Eusocial species are present only in Apidae (4 out of 21, 19.0%) and Halictidae (12 out of 24, 50.0%). The semisocial species is Vestitohalictus vestitus (Halictidae, 4.2%). Kleptoparasitic species are present only in Apidae (4 out of 21, 19.0%), Halictidae (3 out of 24, 12.5%) and Megachilidae (1 out of 25, 4.0%).

Regarding the nesting behaviour, most bees lay their eggs in cavities, either pre-existing or newly excavated (in the ground, in dead plant stems or wood, in snail shells…). Nonetheless, some bee species have different behaviours, such as making the nest with mud (mason bees) or plant fibres and animal hair (carder bees). Finally, kleptoparasitic bees use the nests of other species (Michener, 2007).

Among the 100 species for which we have information about this trait, 70 species are excavators: they make nests in new cavities, either in the ground (65 species), in stems (4) or wood (1). Excavators in stems and wood are all Apidae (5 on 19, 26.3%). Ground excavators are mainly Andrenidae (21 on 21) and Halictidae (31 on 34, 91.2%). There are 19 renter species (that use existing cavities), 3 of which are Megachilidae that use snail shells (3 on 15, 20%). The others are Apidae (4 species, 21.1%), Colletidae (4, 44.4%) and other Megachilidae (8, 53.3%). There are also 2 mason bees (Megachilidae, 13.3%) and a carder bee (*Bombus pereziellus*, Apidae). Finally, 8 species are kleptoparasitic.

All bee species feed on floral resources (nectar or pollen) both at the larval and adult stage (Wilson, 1971; Michener, 2007). Nonetheless, they can show a different degree of specialisation in the plants they feed on (Willmer and Stone, 2004; Michener, 2007): bees are divided into oligolectic species (specialist bees that collect pollen from one plant family) and polylectic (generalist bees that feed on several distantly related plant species). Monolecty (bee species with one host plant) is extremely rare (Willmer and Stone, 2004; Michener, 2007; Danforth et al., 2013). We have data about the lecty of 98 species (the 8 kleptoparasitic species cannot be assigned a lectic status): 22 species are oligolectic (22.4%) and 76 are polylectic (77.6%). Oligolectic species are mainly Andrenidae and Megachilidae (9 species each, 9.2% each). All Halictidae are polylectic.

Finally, bee species can differ in the number of broods they have per year, a trait usually referred to as voltinism (Wilson, 1971; Michener, 2007). Bee species can be univoltine (one brood per year), bivoltine (two broods) or multivoltine.

Among the 108 species for which we have data about voltinism, 90 species (83.3%) are univoltine, 9 are strictly bivoltine (8.3%) and 2 are strictly multivoltine (1.9%). The other species are known to have variable behaviour (one to two broods, one to more than two broods per year). Anyway, data about voltinism are less affordable, since the data we have are, for many species, drawn from observation made in the United Kingdom.

2.5. Body mass and monthly collected community biomass

We measured the dry body mass of all the specimens to measure the total collected biomass of the community and to test the relationship between ITD and body mass. Museum specimens are hardly separable from their entomological pins (Gilbert, 2011): different methods have been proposed to estimate dry body mass without separating each specimen from its pin (Gilbert, 2011; Kendall et al., 2018). In this project, we used a simple method that has some similarities with Kendall's (Kendall et al., 2018): after removing the greatest possible amount of pollen from legs or abdomen with a pin, we weighed each bee with its pin using a Radwag MYA 2.4Y precision microbalance (Radwag Balances and Scales). This microbalance has a sensibility of 10-6 grams. Using the same balance, we measured the mass of pins for each brand and size (see table A2 in the appendices) in order to estimate the pin mass. We calculated pin mass mean and standard deviation for every type of pin. Since the variation was small (e.g., std. dev. = 0.3 mg, relative error < 1% for Kabourek size 1) when compared to intraspecific body mass variation in our sample, we calculated the bee body mass by subtraction of the pin weight on the total weight. For specimens mounted on minutiae, we subtracted the average minutia mass from the total mass; for any other individual, we subtracted the average mass of Kabourek 1. Then we analyzed the relation between this first body mass estimate and the intertegular distance (ITD, as standard size measure) in our sample: we identified outliers and made a precise identification of their pins. Then we subtracted the correct pin mass from the total mass. Finally, an average mass for each species was calculated.

We calculated a total collected biomass for each month by summing up the mass of all bees belonging to the studied species that were caught each month, considering that each bee had a body mass that was identical to specific average body mass.

2.6. Specimen preparation and morphometric measurements

Estimates of ITD and surface-to-volume ratio for each specimen were calculated from the three-dimensional coordinates of a set of landmarks. In order to measure these coordinates, we prepared the specimens: at first, we humidified bees for 48 hours (in glass or plastic alimentary boxes with paper towels soaked in water, closed and refrigerated); then we could easily manipulate them with entomological pinces and pins. Finally, bees were dried at room temperature for at least 48 hours.

Insect bodies are made of three different *tagmata* (head, thorax and abdomen). In many hymenopterans (Hymetopera: Apocrita), including bees, the first segment of abdomen, *propodeum*, contributes to the *mesosoma* along with the three thoracic segments. For convenience, we will refer to the three *tagmata* as to head, thorax and abdomen (instead of head, *mesosoma*, *metasoma*). These body parts can easily have different relative position and orientation. Therefore, each single *tagma* must be treated as a different body when performing morphometrics. Our measurements were performed on head, thorax and the first and second segment of the abdomen. Only two segments are studied for the last *tagma*, because the visible part of segments 3 to 6 can vary greatly due to the telescopic structure of the abdomen. This effect is absent or greatly reduced on the first two segments. We chose a sample of measurements that could give a rough three-dimensional description of head, thorax and T1 and T2 tergites of the abdomen, *i.e.* a measurement of length along the anteroposterior (AP), left-right (LR) and dorsoventral (DV) axes for each *tagma*. To obtain these measurements, we chose a set of 6 anatomical landmarks on the head, 6 on the thorax and 12 on the abdomen (see figure 2.3a and 2.3b). The three-dimensional coordinates of these points were measured with a MicroVu Vertex 251 HC. This optical instrument makes extremely precise measurements, with the possibility to observe small objects with a 20x to 361x zoom: it can be used to select specific points on the surface of an object and to obtain their three-dimensional coordinates with a precision of 10^{-4} millimetres. The 24 points (see figure 2.3a and 2.3b) were taken in 6 times for each bee, from a dorsal and a lateral view for each *tagma*. Lateral measurements were performed on the left side. For a list and description of each landmark and measure, see table A3 (appendices). Landmarks from the same tagma were taken from two different views, so that they could not be easily combined. Hence, we focused on traditional morphometrics. Dimensions of T1 and T2 tergites of abdomen were geometrically calculated from raw data (see table A3). As a result, we obtained 9 measures of length, three for each tagma (H_{AP}, H_{LR}, H_{DV}, T_{AP}, T_{LR}=ITD, T_{DV}, A_{AP}, A_{LR}, A_{DV}, where the first letter stands for the *tagma* – Head, Thorax, Abdomen – and the subscript indicates the axis; note that these traits include ITD). Estimates for body surface (S) and volume (V) were calculated from these 9 traits, approximating bee body as a set of three parallelepipeds (the *tagmata*), the dimensions of which corresponded to the three traits for each *tagma*.

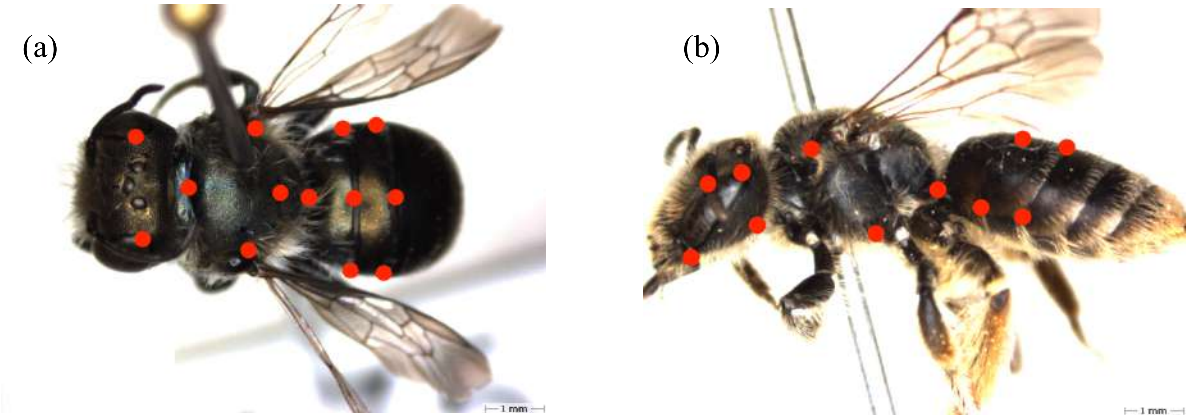

Figure 2.3: (a) dorsal view of *Osmia submicans* with landmarks; (b) lateral view of *Andrena cinerea* with landmarks.

2.7. Hair density

In order to test whether hair density acts as a buffer of temperature effect on body size variation in the community, we measured a proxy of thoracic hair density: we measured the linear hair density on the scutum using the MicroVu Vertex 251 HC. Each bee was observed from a left lateral view at a 250x zoom, looking at the dorsal margin of scutum just above the left tegula. Keeping the upper margin of the thorax horizontal, we performed an autofocus command with the MicroVu and then we counted the hairs that were in focus. At a 250x zoom the area framed by the instrument is about 0.4 mm wide (and constant in each measurement), so that we can compare linear densities in different specimens. In some cases, the measurement was not possible, mainly due to hair degradation or dirt. We were able to perform the linear hair density measurements on 328 individuals from 114 species (see table A2 in the Appendices). Species average hair densities were calculated.

2.8. Tongue length

We used a database of female bees' tongue lengths (proboscis lengths, *i.e.* the sum of the lengths of *prementum* and *glossae*) that were available for a subsample of the specimens used for our morphometric measurements (278 specimens, 95 species) (F. Bastianelli, unpublished data). These measurements were obtained by humidifying bees and dissecting bee mouth parts with entomological pinces; then, mouth parts were mounted on a microscope slide; finally, each tongue specimen was photographed with a Zeiss SteReo Discovery V20 stereo microscope, mounting a Plan S 1.0x FWD 81 mm objective and a Zeiss AxioCam IcC5 camera (Carl Zeiss AG) at iEES in Paris; measurements were taken using ImageJ Software (Rueden et al., 2017) (F. Bastianelli, unpublished data). Average proboscis length was available for 95 species (see table A2 in the Appendices).

2.9. Phylogeny

Bee phylogeny is still widely unsolved and discussed: there is a lack of molecular data for many species and groups, and distances between species are largely unknown (Danforth et al., 2013; Hedtke et al., 2013; Gérard et al., 2018). Simplifying Kendall's approach (Kendall et al., 2018) we constructed an applicable phylogeny for our study, that was based on a maximum likelihood (ML) bee genera tree by Hedtke (Hedtke et al., 2013).

We rooted the tree following the available information on bee families' phylogenetic relationship (Danforth et al., 2013; Hedtke et al., 2013), and we ultrametriced it with *ape* function *makeChronosCalib* (with *node=root*; *age.max* = 130). We pruned the genera that were not observed in our community. Then we added the branches representing the bee species belonging to different genera. Since we did neither know the distances between each couple of species, nor the structure of the phylogeny at the genus level, we added all the species belonging to a single genus in a politomy. We arbitrarily imposed a length of terminal branches equal to the 75% of the length of the shortest terminal branches in the backbone tree (*Hylaeus* and *Colletes* branches). Phylogenetic data were manipulated with *ape* (version 5.1, Paradis et al., 2004) and *phytools* (version 0.6.44, Revell, 2012). The tree we obtained contained 120 species. 1 species (*Nomiapis diversipes*), the genus of which (1 out of 29) was not present in the backbone tree, was excluded.

2.10. Environmental temperature data

To obtain an estimate of flight temperature for each specimen of the collection, we used the public database of the European Climate Assessement and Dataset (ECAD 17.0). This dataset consists of temperature and precipitation data on the European climate obtained from "daily station series from climatological divisions of National Meteorological and Hydrological Services and station series maintained by observatories and research centres throughout Europe and the Mediterranean" (Haylock et al., 2008). We used the dataset of maximum daily temperature, provided with a spatial resolution of 0.25 degrees in latitude and longitude. Data were extracted using *climateExtract* (version 1.17.0, Schmucki) using coordinates of the seven study sites. To estimate the flight temperature of each bee, we calculated the average daily maximum temperature of the week that preceded and included the day of the collection. This way each specimen of the collection was associated with an observed flight temperature.

2.11. Data analysis

All analyses were performed on R 3.4.1 (R Core Team, 2017). We used the packages: *ape* (version 5.1, Paradis et al., 2004), *caper* (version 1.0.1, Orme et al., 2018), *car* (version 3.0.2, Fox and Weisberg, 2011), *ctv* (version 0.8.5, Zeileis, 2005), *climateExtract* (version 1.17.0, Schmucki), *devtools* (version 1.13.6, Wickham et al., 2018), *kimisc* (version 0.2.1, Mueller, 2014), *geiger* (version 2.0.6, Harmon et al., 2008), *methods* (version 3.4.1, R Core Team, 2017), *nlme* (version 3.1.131, Pinheiro et al., 2017), *phylobase* (version 0.8.4, R Hackathon et al., 2017), *phylosignal* (version 1.2, Keck et al., 2016), *phytools* (version 0.6.44, Revell, 2012), *picante* (version 1.7, Kembel et al., 2010), *plyr* (version 1.8.4, Wickham, 2011), *scales* (version 0.5.0, Wickham, 2017a), *stringr* (version 1.2.0, Wickham, 2017b), *vioplot* (version 0.2, Adler and Adler, 2005).

In many analyses we performed several kinds of linear models (ordinary least squares, mixed-effect models and phylogenetic least squares models). In all cases, models assumptions were checked visually.

2.11.1. Seasonal diversity patterns

We observed the variation in presence and abundance for each species and family along the year. Since the collection protocol had a monthly basis, we chose the month as the smallest time unit in our analyses: then, we computed the monthly observed abundance of each species. We calculated the observed species richness and Simpson's diversity index (D) (Simpson, 1949) for each month.

To estimate the seasonal trend of diversity taking into account phylogenetic relationships, we calculated the standardized effect size mean pairwise distance (SES_{MPD}) index (either abundance weighted or not) for each month using *picante* on the 119-species subsample (excluding *Apis mellifera* and *Nomiapis diversipes*) (Webb et al., 2002; Kembel, 2009; Kembel, 2010; Kembel et al., 2010). Mean pairwise distance (MPD) corresponds to the mean phylogenetic distance between two randomly chosen species (or individuals, if abundance weighted) (Webb et al., 2002; Kembel, 2009; Kembel, 2010). SES_{MPD} evaluates the difference between the observed MPD of a community and a distribution of pseudo-communities generated with a randomization method (Webb et al., 2002; Kembel, 2009; Kembel, 2010). In our case, we used the simplest randomization method (shuffling the tips of the trees) for 10 000 runs (Kembel, 2009; Kembel, 2010).

The honeybee (*Apis mellifera*) is present all along the year in our collection. Nonetheless, the abundance of this species in the community is certainly highly influenced by beekeeping practices. One of these practice is colony seasonal migration (or transhumance), *i.e.* the translocation of colonies to protected or mountain areas in summer, which is necessary to guarantee colony sustainability for beekeeping (Henry & Rodet, 2018). In Corsica, most colonies are moved to the mountains in summer (A. Perrard, personal communication). This means that, in summer, the abundance of this species in the territory of Bonifacio is artificially reduced. Since our goal is to study the effects of some ecological variables on size and shape patterns in the community, we performed all the models on a subset of our collection data excluding honeybees, unless stated otherwise.

2.11.2. Size and shape variation

We performed a principal component analysis (PCA) on the dataset of measurements for each bee (391 individuals, 121 species). At first, the 9 measures were linearized by calculating their natural logarithm, then a scaled PCA was performed. This way we obtained 9 different principal components. In this case, first principal component (PC1) is considered to describe all isometric and allometric variation with size (Jolicœur and Mosimann, 1960).

We performed a multivariate analysis of variance (MANOVA) on the PCs dataset, using species as groups, in order to test whether intraspecific variation was significantly smaller than the interspecific one. We performed the MANOVA both on the complete PCs dataset and on a reduced dataset, excluding PC1: if intraspecific variation proves to be significantly smaller than interspecific in both cases, we can infer that our measurements highlight the presence of shape variation among different species, and that this variation is not only caused by size.

2.11.3. Allometric trends

We tested the presence of static allometry (also called interspecific or phylogenetic allometry) in the community, to understand whether size variation is correlated with shape variation. An allometric trend is defined as a relationship between two different quantitative traits following the form:

$$Y = aX^b$$

With $b \neq 1$ (the case $b = 1$ corresponds to an isometric trend).
The relationship can be expressed in the logarithmic form:

$$\log(Y) = \log(a) + b\log(X)$$

We performed several linear models to evaluate the relation between ITD and all other morphometric traits. We chose ITD as effect variable (as it is the standard size measure for bees) (Osorio-Canadas et al., 2016) and performed ordinary least squares (OLS) models with the *nlme* form:

$$\log(Y) \sim \log(ITD)$$

with Y being one of the other 8 variables, and ~ being the symbol used in *nlme* to express that the response variable (on the left) is linearly modelled relative to the effect variables (on the right). In this case, the trend is considered allometric if the regression coefficient is significantly different from 1. Models were performed on the species average values, with species divided into 5 groups, corresponding to the 5 observed families (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae). We performed type II analyses of variance (ANOVAs) to test whether the relationships were significant (*i.e.* linear coefficient significantly different from 0), and computed a 95 % confidence interval to test whether the regression coefficient was significantly different from 1 (isometry).

2.11.4. Relationship between body mass, volume, ITD and S/V

We performed simple linear models on couples of variables (average values of body mass M, body volume V, ITD and S/V ratio for each species), with or without a family fixed-effect. These models were performed on the species average values of morphological traits.

The models we performed were:

a) V ~ M (if V is a good estimation of body volume and body density variation is limited in the community, we expect to see a strong linear relationship between V and M);
b) log (M) ~ log (ITD) (to test whether ITD is a good proxy of body mass); we expect a strong relationship between these variables (ITD is widely used as a proxy of bee body mass) (Cane, 1987; Kendall et al., 2018);
c) log (S/V) ~ log (ITD) (we expect a negative relationship between these two variables, as volume generally increases with body mass, if density is constant, and S/V decreases with volume; nonetheless, if variation in shape is important inside the community, we expect that an important part of S/V variation is not explained by ITD).

2.11.5. Seasonal trends of size and shape

We performed a linear fixed-effect model selection to describe the seasonal variation of ITD and S/V ratio. As we observed higher S/V ratio and smaller ITD in summer, we separately fitted these two variables with a function of time (expressed in months), and a family fixed effect, with interaction between time and family. These models were performed on the whole collection dataset, considering that each bee morphological traits were equal to the average specific values. This means that bees of the same species were considered to have the same body mass, size and shape, but differed in the date of collection and the observed flight temperature. This assumption allows us to take into account specific abundances.

We performed models of the *nlme* form:

$$\text{ITD} \sim \text{month*Family+month}^2\text{*Family+month}^3\text{*Family}$$
$$\text{S/V} \sim \text{month*Family+month}^2\text{*Family+month}^3\text{*Family}$$

Family stands for the family fixed effect. We expect ITD and S/V to follow seasonal variation of environmental temperature, that is cyclic, with lower values both at the beginning and at the end of the season. Therefore, we want a function of the second degree, at least. We tested a 3rd-degree function to allow the model to describe a more complicated trend, *e.g.* with different concavity in the first and last parts of the season, or with more than one stationary point. Then we performed mixed-effect models adding a random effect of collection-site to the last two models:

$$\text{ITD} \sim \text{month*Family+month}^2\text{*Family+month}^3\text{*Family + random =}\sim 1|\text{Site}$$

$$\text{S/V} \sim \text{month*Family+month}^2\text{*Family+month}^3\text{*Family + random =}\sim 1|\text{Site}$$

The notation *random* = ~1|*Site* stands for a random effect of collection-site.

Fixed-effect model and mixed-effect linear model were compared using type II ANOVA, to identify the best fitting models.

After identifying the best model for both response variables, we performed a model selection analysis on the 5 subsamples corresponding to families (excluding *A. mellifera* in Apidae), to identify the best model that described the pattern of variation of each family.

2.11.6. Test of Bergmann's hypothesis: comparative method

Bergmann's thermoregulation hypothesis predicts a smaller surface-to volume ratio with lower temperatures (Bergmann, 1847). To test this hypothesis using a cross-species approach (Osorio-Canadas et al., 2016), we need flight temperature proxies for the species of the community. We calculated two different estimates of flight temperature for each species: first, the minimum observed flight temperature; second, the average observed flight temperature. The minimum flight temperature of each species is considered to have a greater informative value than the maximum, as high temperature is generally less limiting for flying (Peters et al., 2016). Nonetheless, estimating an affordable value of minimum flight temperature can be hard, especially for rare species: the first observation of a species may happen with a variable delay on each species' first flight. On the other side, average temperature should be less prone to this kind of stochasticity, and then it should be more easily comparable between species.

We performed linear fixed-effect models to describe the relationship between ITD (or S/V) and the two estimates for species flight temperature. The models had the *nlme* form:

$$\log(\text{ITD}) \sim \log(\text{T})\text{*Family}$$

$$S/V \sim T*Family$$

with T being either the minimum or the average observed species flight temperature. The first model is in the logarithmic form as in previous literature (*e.g.* Osorio-Canadas et al., 2016). We also tested a family fixed-effect. We performed ANOVAs to test for the significance of effects.

We estimated the phylogenetic signal of our variables, in order to determine whether previous results could be influenced by the phylogeny of the species. Phylogenetic signal is a “tendency of related species to resemble each other [in a trait] more than species drawn at random from the same tree” (Münkemüller et al., 2012). We calculated Pagel’s λ (estimated by ML) (Pagel, 1999) for ITD, S/V and both temperature estimates with *phylosignal*. Pagel’s λ describes the transformation of the phylogeny required so that the trait data fit best with a Brownian motion model of evolution (Münkemüller et al., 2012). When the phylogenetic signal was significant, we took the phylogenetic non-indipendence of the taxa into account using phylogenetic least squares (pGLS) analysis with *ape* and *nlme*. We tested relationship of the same form of the previously introduced models: relationship between ITD (or S/V) and minimum temperature (or average temperature). In pGLS analyses we introduced either a Brownian motion model of evolution (with *corBrownian* function), simulating pure drift evolution, or an Ornstein-Uhlenbeck model (with *corMartins* function, with $\alpha_0 = 0.001$) simulating evolution by natural selection and drift (Butler and King, 2004). As for the previous models, the effect of temperature was evaluated with ANOVAs. Phylogenetic comparative analyses (Pagel’s λ and pGLS) were performed on the 119-species dataset.

2.11.7. Test of mechanistic hypotheses: community approach

We also used a community wide approach, to test some predictions made by Bergmann’s thermoregulation hypothesis (Bergmann, 1847) and the resource abundance hypothesis (Watt et al., 2010). The latter predicts a positive relationship between size and resources (Watt et al., 2010). We made the hypothesis that if Bergmann’s hypothesis is valid, we should observe a negative relation between temperature and the community average value of ITD (or a positive relationship with S/V): we expect that with low temperatures the community should be composed of bigger bees and hence, that bee species with higher average ITD should be more abundant. On the other side, if food availability has a positive effect on body size, we should observe a positive relationship between the community average ITD and resource abundance (negative with S/V): this means that with more resources, bee species with higher average ITD should be more abundant (for convenience, we called this prediction the resource-abundance hypothesis).

To test these hypotheses, we performed a linear mixed-effect model on ITD (or S/V) including total monthly biomass (B_m) and observed flight temperature

(T) as explicative variables. We considered the monthly collected biomass of bees as a proxy of resource abundance (see 3.2). Finally, we added a random effect of collection-site:

$$ITD \sim scale(T) + scale(B_m) + random = \sim 1|Site$$

$$S/V \sim scale(T) + scale(B_m) + random = \sim 1|Site$$

Both temperature and biomass are scaled to a distribution of mean and standard deviation equal to 1 (notation *scale(effect)*), so that both effects are comparable.

We performed these models on the five subsamples of the collection dataset corresponding to the five families. This approach is equivalent to study the relationships of each family's weighted mean of ITD (or S/V) with both temperature and biomass.

We performed marginal (type III) ANOVAs to test whether each effect was significant, and ANOVAs to compare different models. We tested the relative importance of the relationship between ITD (or S/V) and temperature, and ITD (or S/V) and resources by calculating the beta coefficients: this was possible, since analyses were performed on scaled values of temperature and biomass.

2.11.8. Hair density effect

We also tested the effect of thorax hair density on S/V. At first, we repeated the same models introduced in paragraph 2.11.7 on the reduced dataset containing the 113 species for which hair density data were available. Then, we calculated the residuals of $S/V \sim T + B_m$ models and analysed the relationship between these residuals and hair density (H) with a linear model for each family:

$$Res(S/V \sim T+B_m) \sim H$$

Where *Res(model)* stands for the residuals of the model.

We hypothesize that bees with higher hair density can fly at a lower environmental temperature when compared to other bees of the same size, since their thermoregulation effort should be reduced. Hence, at a given temperature, we expect that a higher hair density could explain a higher than average S/V (a higher residual). Therefore, we expect a positive relationship.

2.11.9. Tongue length variation

To understand the possible effects of body size evolution on the tongue size variation in the community, we studied the proboscis length (PL).

At first, we studied the allometric relationship between ITD and proboscis length (PL) in our community by performing a model of the form:

$$\log (PL) \sim \log (ITD) * Family$$

Each effect was tested with a type II ANOVA. We wanted to test whether the known allometric trend between PL and ITD is verified in our community (Cariveau et al., 2016).

To study the variation in PL along the season with a community approach, we performed a model similar to the models introduced in paragraph 2.11.5:

$$PL \sim month*Family+month^2*Family+month^3*Family + 1|Site$$

and conducted similar analyses. We expect to find a seasonal trend similar to the one observed for body size (ITD), with longer tongues in the colder months or when resources are the most abundant.

3. RESULTS

3.1. Seasonal diversity patterns

Species richness (for the community and for each family), absolute abundances of the 5 families and the community and Simpson's D index are reported in table 3.1. The relative abundances of different families in the community along the season are represented by a stacked bar plot (figure 3.1a including *A. mellifera*, 3.1b excluding *A. mellifera*).

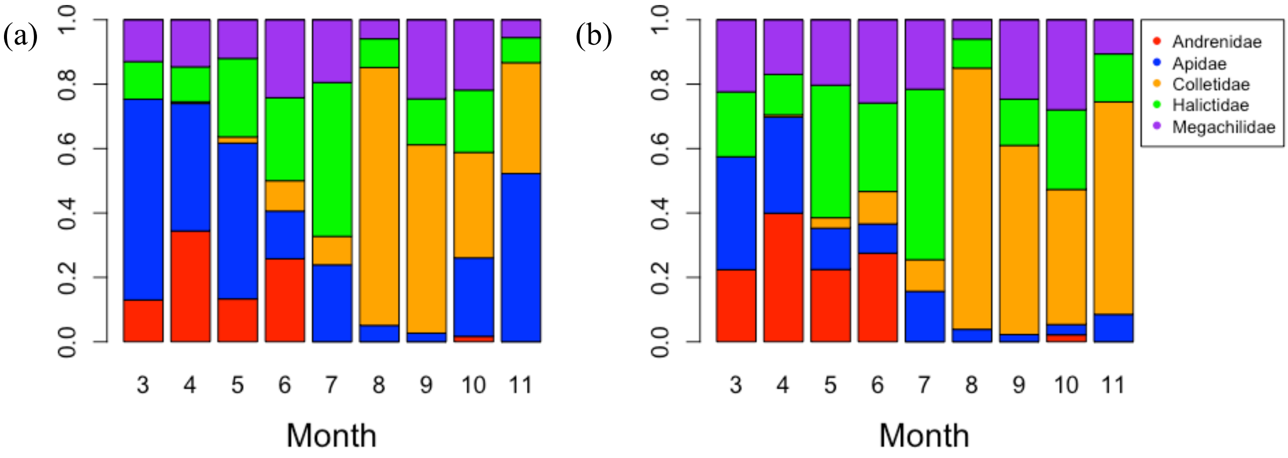

Figure 3.1: monthly community composition in families, a) including or b) excluding *A. mellifera*

We observe that the community composition in families varies along the season. Andrenidae are not present in July, August, September and November; Colletidae are not present in March. All other families are present all along the season. The relative and absolute abundances of families show important changes, *e.g.* we observe that in the first part of the season (from March to June) Colletidae are rare or absent, while they become the dominant group from August to November. Excluding *A. mellifera* leads to important changes in Apidae relative abundance in the first and the last parts of the season, as this species is the most occurring in the group in these periods (up to 84 % of Apidae individuals in the same month in spring, 91% in autumn).

Simpson's D index corresponds to the probability that two randomly chosen individuals belong to the same species in a community (Simpson, 1949). We observe that this probability varies along the season, with maximum values in August (50.5%) and September (21.9%). In the other months, D is lower, (5-14%), with the lowest value observed in May (4.6%).

Table 3.1: monthly species richness (R) and absolute abundance (A) for each family and for the whole community; Simpson's diversity index (D). *A. mellifera* is excluded.

	Andrenidae		Apidae		Colletidae		Halictidae		Megachilidae		Community		
Month	R	A	R	A	R	A	R	A	R	A	R	A	D
3	7	30	7	47	0	0	6	27	2	30	22	134	0.115
4	13	127	8	95	1	2	15	40	10	54	47	318	0.072
5	11	42	8	24	3	6	21	77	11	38	54	187	0.047
6	4	115	10	38	6	42	20	115	11	108	51	418	0.109
7	0	0	7	16	4	10	11	54	6	22	28	102	0.076
8	0	0	4	9	5	189	6	21	4	14	19	233	0.506
9	0	0	5	5	8	131	12	32	7	55	32	223	0.219
10	2	2	2	3	4	39	7	23	6	26	21	93	0.093
11	0	0	2	4	6	31	3	7	3	5	14	47	0.144

Phylogenetic diversity values along the season (expressed by $\mathrm{SES}_{\mathrm{MPD}}$ and computed on the 119-species dataset for which we have an applicable phylogeny) are reported in table 3.2.

Table 3.2: Phylogenetic diversity (estimated as MPD and $\mathrm{SES}_{\mathrm{MPD}}$) along the season.
N_{species} = number of species; MPD = observed MPD; SES = standardized effect size; p = p-value

		Non-weighted			Abundance-weighted		
Month	N_{species}	MPD	$\mathrm{SES}_{\mathrm{MPD}}$	p	MPD	$\mathrm{SES}_{\mathrm{MPD}}$	p
3	22	156.34	-0.40	0.27	147.55	0.73	0.76
4	47	156.37	-0.79	0.19	147.32	0.05	0.43
5	54	154.04	-1.96	0.04	146.60	-0.91	0.16
6	51	150.25	-3.42	0.01	151.66	1.24	0.95
7	28	145.20	-3.14	0.01	126.04	-2.94	0.02
8	18	150.26	-1.33	0.10	57.83	-2.02	0.04
9	32	145.06	-3.61	0.01	108.97	-1.29	0.11
10	21	153.38	-0.95	0.15	131.79	-1.34	0.09
11	14	145.60	-1.65	0.07	104.15	-2.86	0.02

For non-abundance-weighted MPD, we observe that the $\mathrm{SES}_{\mathrm{MPD}}$ is always negative. Negative $\mathrm{SES}_{\mathrm{MPD}}$ values and low p-values (<0.05) are usually interpreted as a signal of tree-wide patterns of clustering (Kembel, 2009; Kembel, 2010), *i.e.*, species of our community are clustered in the phylogeny or species distances are significantly lower than expected. In our case, this would mean that species present in a given month tend to be phylogenetically close. We observe that this happens from June to September and in November. These changes cannot be due only to changes in species richness (see the difference between May and June). On the other end, weighted SES_{MPD} highlights a more complicated pattern. We have positive SES_{MPD} values in March and June, meaning that individuals observed in these months tend to belong to different, phylogenetically distant, species. Significantly negative values are observed from July to November.

3.2. Total collected biomass

We estimated the total biomass of all the female bees of the studied species that were collected each month (see table 3.3 for the results including or excluding *A. mellifera*). The trend is represented compared to a trend of flower abundance in a Mediterranean community (figure 3.2a and 3.2b respectively) (Flo et al., 2018).

Table 3.3: estimate of the total collect biomass for each month, including (B_{mA}) or excluding *A. mellifera* (B_m). The latter is the one used for the analyses.

Month	B_{mA} (mg)	B_m (mg)
3	9078.872	6011.965
4	13874.900	12262.402
5	8445.072	4366.401
6	9072.244	8816.951
7	2041.057	1693.263
8	1075.141	980.289
9	1668.617	1636.999
10	1736.705	914.647
11	2090.632	731.076

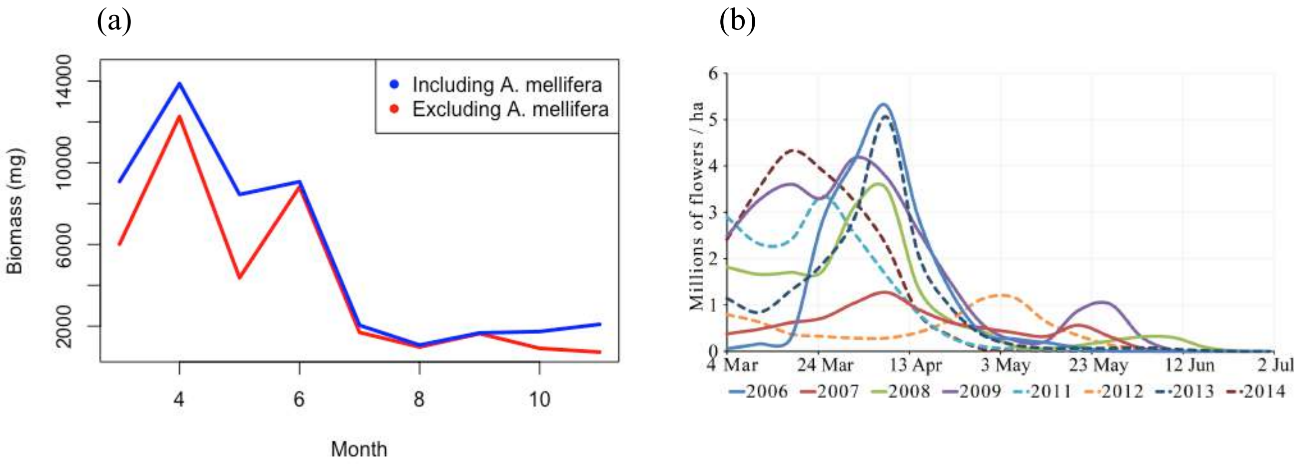

Figure 3.2: (a) trend of the total collected biomass estimate in Bonifacio including or excluding *A. mellifera*; (b) trend of flower abundance from March to July for 23 flower plant species in a Mediterranean community in Spain: different lines represent different years (from Flo et al., 2018).

We observe that the total biomass of bees is higher in the first half of the flight season. The total biomass (excluding *A. mellifera*) reaches its maximum values in April and June, with a loss of about 30% of the total biomass between the two. We observe an important reduction of bee biomass in July. Biomass remains low for all the rest of the season. When we consider *A. mellifera*, we observe significantly higher biomass from March to May, and in the last two months. In certain months, honeybee biomass covers an important percentage of the community biomass (48% in May, 65% in November). Honeybees are overall absent from June to September, when beekeepers move their hives to the mountains. We observe that our trend in biomass has some similarities with the trend of flower abundance in a Mediterranean Spanish community (Flo et al., 2018). Similar trends were observed for sugar content in nectar (Flo et al., 2018). The flower abundance trend seems to be translated, compared to our biomass trend. We do not have data about flower abundances in Bonifacio, but, if the trend is similar to the one observed in Spain, we suggest that total bee biomass can be used as a proxy for the abundance of floral resources in a community in further analyses.

3.3. Size and shape variation

For the PCA performed on the 391-individuals dataset, the first principal component, PC1, explains 92.56% of the variance (see table 3.4).

Table 3.4: proportion of variance explained by each principal component. PC = principal component; EV = variance explained by the PC

PC	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
% EV	92.56	2.97	2.74	0.95	0.82	0.37	0.28	0.20	0.13

The first two PCs show a strong variation within families, with both extremes along PC1 being members of Apidae family (see figure 3.3). Families seem to be more clustered along PC2.

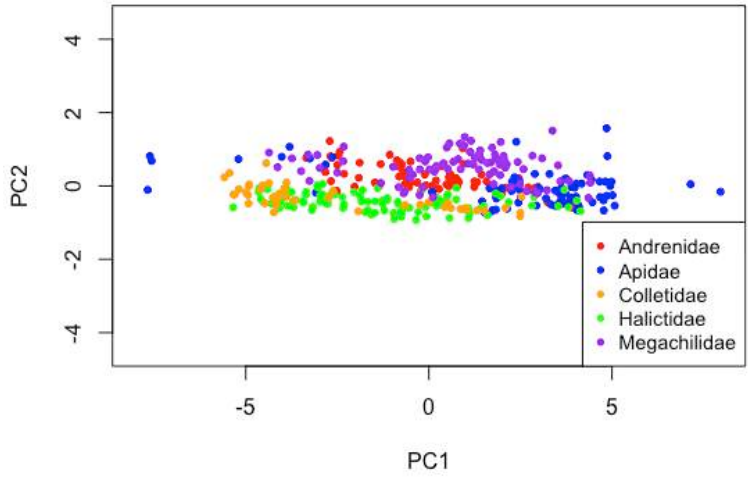

Figure 3.3: plot of PC1 and PC2

MANOVAs yielded a significant difference between inter- and intraspecific morphological variation, both including (Df=120, F = 8.132, $p < 0.0001$) and excluding PC1 (Df=120, F = 7.052, $p < 0.0001$). This means that intraspecific variation is significantly smaller than the interspecific in our sample.

3.4. Allometric trends

Analyses about allometries are reported in table 3.5. We observe that different families show different allometric trends. Except for Halictidae, head traits show an overall negative allometry with ITD. This means that in most families, bigger species have smaller heads, relative to ITD, than smaller species. Halictidae show a thicker, shorter head in bigger species. Thorax traits are isometric in Andrenidae and Halictidae, and hence, proportions are overall maintained in the thorax in these groups. Colletidae show negative allometries for both traits, and Apidae and Megachilidae show an isometric trend for one trait (T_{AP}) and a negative allometry for the other (T_{DV}). Hence, larger Colletidae species have shorter and flatter thoraces, while Apidae and Megachilidae seem to show thinner thoraces, relative to ITD, in larger species. Abdomen traits seem to be isometric in Andrenidae and Halictidae. Larger Apidae species show wider abdomens, while Colletidae and Megachilidae show stouter abdomens in bigger species.

Table 3.5: allometry analyses per family, including the result of the test with the 95 % confidence interval: traits for which the regression coefficient is significantly different from 1 (*i.e.* 1 is not comprised in the confidence interval, column Allometry) are marked with + (slope >1, positive allometry with ITD) or – (slope<1, negative allometry). Non-significant allometric trends are marked with 0 (slope≈1, considered as isometry). Df = degrees of freedom; F = F-test value; m = regression coefficient; sd_m = m standard deviation.

Andrenidae

Trait	**Df**	**F**	**p**	**m**	**sd_m**	**Allometry**
H_{AP}	19	307.68	3.41e-13	0.92	0.05	0
H_{LR}	19	297.09	4.67e-13	0.84	0.05	–
H_{DV}	19	258.02	1.64e-12	0.88	0.05	–
T_{AP}	19	486.73	5.31e-15	1.02	0.05	0
T_{Dv}	19	372.34	6.10e-14	0.96	0.05	0
A_{AP}	19	183.21	3.31e-11	0.98	0.07	0
A_{LR}	19	282.95	7.22e-13	1.10	0.07	0
A_{DV}	19	45.86	1.81e-6	1.2	0.2	0

Apidae

Trait	**Df**	**F**	**p**	**m**	**sd_m**	**Allometry**
H_{AP}	23	827.70	<0.0001	0.85	0.03	–
H_{LR}	23	199.02	8.21e-13	0.74	0.05	–
H_{DV}	23	324.03	4.74e-15	0.72	0.04	–

T_{AP}	23	671.79	<0.0001	1.00	0.04	0
T_{Dv}	23	383.92	7.56e-16	0.89	0.05	–
A_{AP}	23	141.87	2.57e-11	0.99	0.08	0
A_{LR}	23	1118.00	<0.0001	1.09	0.03	+
A_{DV}	23	100.49	7.30e-10	0.89	0.09	0

Colletidae

Trait	**Df**	**F**	**p**	**m**	**sd_m**	**Allometry**
H_{AP}	10	336.60	4.98e-09	0.75	0.04	–
H_{LR}	10	592.3	3.13e-10	0.77	0.03	–
H_{DV}	10	252.09	2.02e-08	0.73	0.05	–
T_{AP}	10	338.03	4.88e-09	0.84	0.05	–
T_{Dv}	10	661.89	1.81e-10	0.92	0.04	–
A_{AP}	10	167.45	1.43e-07	0.70	0.05	–
A_{LR}	10	452.03	1.18e-09	1.03	0.05	0
A_{DV}	10	162.17	1.67e-07	1.09	0.09	0

Halictidae

Trait	**Df**	**F**	**p**	**m**	**sd_m**	**Allometry**
H_{AP}	34	433.47	<0.0001	0.85	0.04	–
H_{LR}	34	206.48	5.26e-16	1.01	0.07	0
H_{DV}	34	341.39	<0.0001	1.15	0.06	+
T_{AP}	34	429.97	<0.0001	1.03	0.05	0
T_{Dv}	34	2238.70	<0.0001	1.03	0.02	0
A_{AP}	34	344.17	<0.0001	1.04	0.06	0
A_{LR}	34	770.89	<0.0001	1.06	0.04	0
A_{DV}	34	404.27	<0.0001	1.08	0.05	0

Megachilidae

Trait	**Df**	**F**	**p**	**m**	**sd_m**	**Allometry**
H_{AP}	25	379.25	<0.0001	0.85	0.04	–
H_{LR}	25	359.12	2.40e-16	0.83	0.04	–
H_{DV}	25	58.14	5.58e-08	0.8	0.1	–
T_{AP}	25	322.77	8.35e-16	0.94	0.05	0
T_{Dv}	25	515.36	<0.0001	0.90	0.04	–
A_{AP}	25	26.68	2.43e-05	0.7	0.1	–
A_{LR}	25	504.23	<0.0001	1.01	0.05	0
A_{DV}	25	34.28	4.16e-06	0.8	0.1	0

3.5. Relationships between mass, volume, ITD and S/V

Linear fixed-effect models yielded significant positive relationship between body volume and body mass and between the logarithms of body mass and ITD. There was a significant negative relationship between log (S/V) and log(ITD). The results were similar both with and without a family fixed effect, and interactions between family and the other explicative variables are always significant (see table 3.6 for details, figure 3.4a, 3.4b and 3.5 for plots of data and model predictions). ITD is a good predictor of body mass (M) and S/V. Estimated body volume (V) seems to be proportional to body mass, but the proportionality depends on the family.

Table 3.6: results of type II ANOVAs of fixed-effect linear models. Df = degrees of freedom; F = F-test value; p = p-value

<table>
<tr><td colspan="4">V~M (Multiple $R^2 = 0.970$; Adjusted $R^2 = 0.970$)</td></tr>
<tr><td>Effect</td><td>Df</td><td>F</td><td>p</td></tr>
<tr><td>M</td><td>1</td><td>3904.50</td><td>< 0.0001 ***</td></tr>
<tr><td colspan="4">V~M*Family (Multiple $R^2 = 0.978$; Adjusted $R^2 = 0.976$)</td></tr>
<tr><td>Effect</td><td>Df</td><td>F</td><td>p</td></tr>
<tr><td>M</td><td>1</td><td>3677.22</td><td>< 0.0001 ***</td></tr>
<tr><td>Family</td><td>4</td><td>5.15</td><td>0.0008 ***</td></tr>
<tr><td>M:Family</td><td>4</td><td>4.25</td><td>0.003 **</td></tr>
<tr><td colspan="4">log(M)~log(ITD) (Multiple $R^2 = 0.951$; Adjusted $R^2 = 0.951$)</td></tr>
<tr><td>Effect</td><td>Df</td><td>F</td><td>p</td></tr>
<tr><td>log(ITD)</td><td>1</td><td>2315.70</td><td>< 0.0001 ***</td></tr>
<tr><td colspan="4">log(M)~log(ITD)*Family (Multiple $R^2 = 0.964$; Adjusted $R^2 = 0.964$)</td></tr>
<tr><td>Effect</td><td>Df</td><td>F</td><td>p</td></tr>
<tr><td>log(ITD)</td><td>1</td><td>1984.17</td><td>< 0.0001 ***</td></tr>
<tr><td>Family</td><td>4</td><td>4.30</td><td>0.003 **</td></tr>
<tr><td>log(ITD):Family</td><td>4</td><td>5.46</td><td>0.0005 ***</td></tr>
<tr><td colspan="4">log(S/V)~ log(ITD) (Multiple $R^2 = 0.981$ Adjusted $R^2 = 0.980$)</td></tr>
<tr><td>Effect</td><td>Df</td><td>F</td><td>p</td></tr>
<tr><td>ITD</td><td>1</td><td>6006.10</td><td>< 0.0001 ***</td></tr>
<tr><td colspan="4">log(S/V)~ log(ITD)*Family (Multiple $R^2 = 0.987$; Adjusted $R^2 = 0.986$)</td></tr>
<tr><td>Effect</td><td>Df</td><td>F</td><td>p</td></tr>
<tr><td>ITD</td><td>1</td><td>5758.15</td><td>< 0.0001 ***</td></tr>
<tr><td>Family</td><td>4</td><td>9.73</td><td>8.1 e-7 ***</td></tr>
<tr><td>ITD:Family</td><td>4</td><td>3.33</td><td>0.01*</td></tr>
</table>

Legend of significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

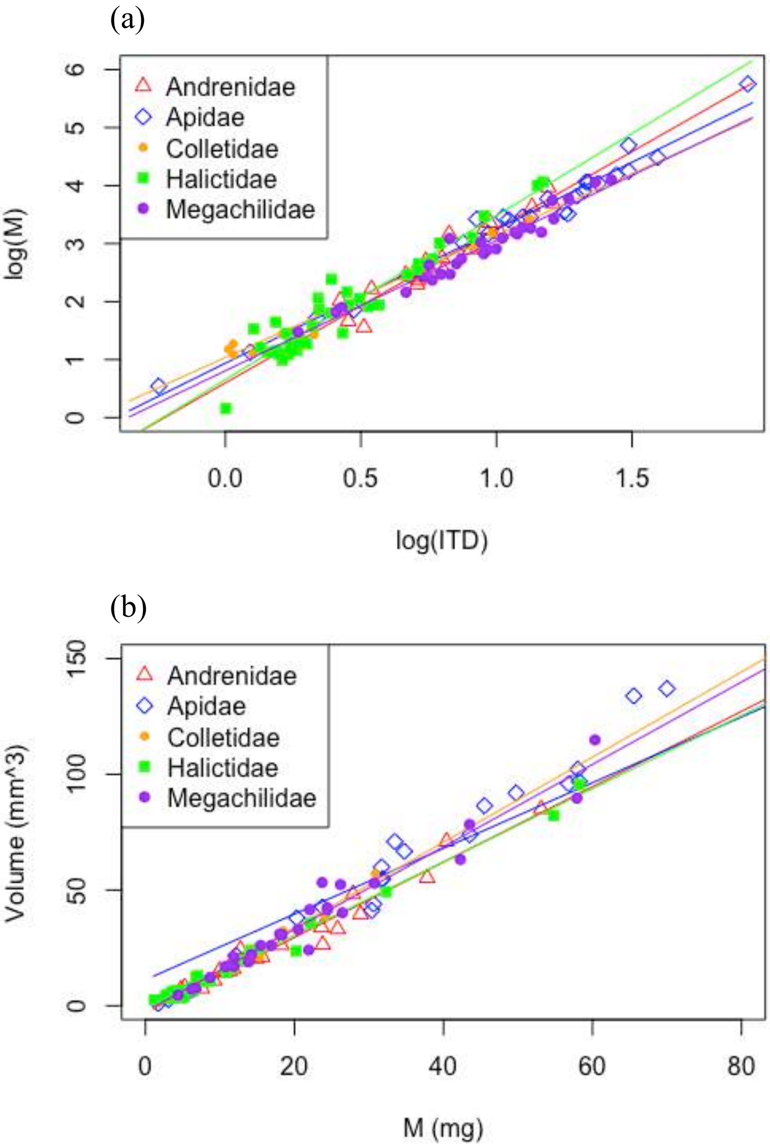

Figure 3.4: (a) plot of log(M) and log(ITD); (b) plot of volume and mass; with model prediction.

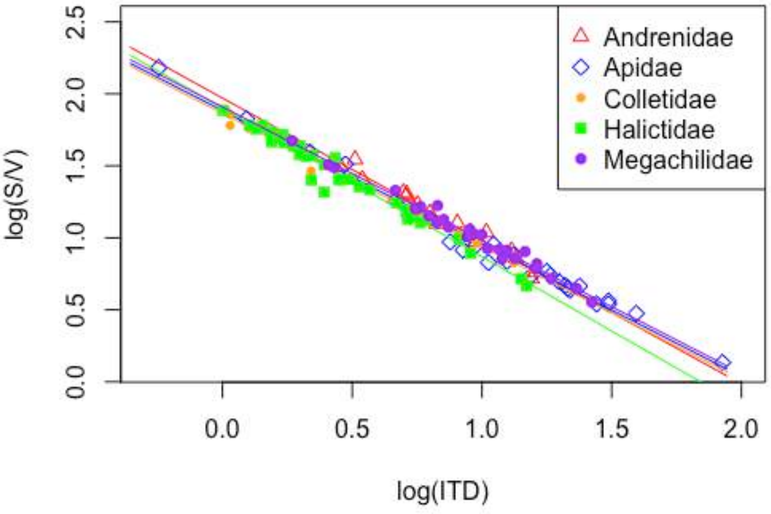

Figure 3.5: plot of log(S/V) and log(ITD) with model prediction.

3.6. Seasonal trends of size and shape

The variation of the distribution of bee body size (ITD) along the season is represented in a violin plot (figure 3.6a and 3.6b including or excluding *A. mellifera*). This type of plot is the combination of a box plot and a density plot, representing at the same time the distribution of the data, the median and the 95% confidence interval.

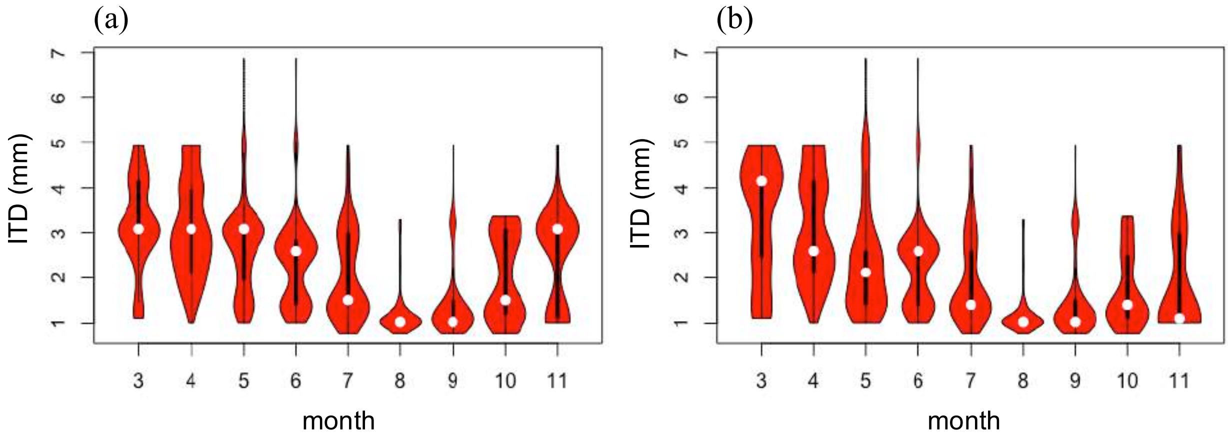

Figure 3.6: Violin plot of the distribution of ITD along the season (a) including and (b) excluding *A. mellifera*.

We observe that in both cases the community tends to have lower ITD values in summer, and higher values in spring and autumn. The presence of *A. mellifera* seems to have an important effect on the distribution in the first and the last parts of the season, with evident big groups with ITD of about 3 mm (the average for *A. mellifera* in our sample is 3.081 mm) in March, April and May and again in October and November. When we observe the pattern without *A. mellifera* we observe that, starting from March toward August, the biggest group of bees that has an ITD of about 4 mm disappears in May-June, while the bees of average size (ITD ≈ 3 mm) almost disappear in August-September, when the community is almost composed only of small bees (ITD ≈ 1 mm or less). The trend is partially inverted in the last part of the season.

Model selection with ANOVA shows that the there is a significant random collection-site-effect both for ITD and S/V (ITD: L ratio = 17.99 $p<0.0001$; S/V: L ratio = 40.40 $p<0.0001$). For all these response variables, there is a significant 3rd-degree relationship with time (expressed in months) and both a fixed family-effect and interactions between family and time are significant (see table 3.7).

Table 3.7: marginal ANOVA results for ITD and S/V seasonal variation. m = time (months); f = family; Df = degrees of freedom; F = F-test value; p = p-value

$ITD \sim m*f+m^2*f+m^3*f+1\|Site$				$S/V \sim m*f+m^2*f+m^3*f+1\|Site$			
Effect	Df	F	p	Effect	Df	F	p
m	1	15.04	0.0001***	m	1	13.67	0.0002***
f	4	22.63	<0.0001***	f	4	15.34	<0.0001***
m^2	1	15.99	0.0001***	m^2	1	14.43	0.0002***
m^3	1	16.05	0.0001***	m^3	1	14.24	0.0002***
m:f	4	25.34	<0.0001***	m:f	4	16.27	<0.0001***
m^2:f	4	26.72	<0.0001***	m^2:f	4	17.40	<0.0001***
m^3:f	4	26.49	<0.0001***	m^3:f	4	17.21	<0.0001***

Legend of significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Model selection on families subsets showed that, both for S/V and ITD the best model is a 3rd-degree function of time in the case of Andrenidae and Apidae, and a 2nd-degree function for other families.
The results of marginal ANOVAs for each family are reported in table 3.8.

Table 3.8: marginal ANOVA results for ITD and S/V seasonal variation models for each family m = time (months); Df = degrees of freedom; F = F-test value; p = p-value

Andrenidae

ITD				S/V			
Effect	Df	F	p	Effect	Df	F	p
m	1	12.62	0.0004***	m	1	10.26	0.001**
m^2	1	12.67	0.0004***	m^2	1	10.09	0.002**
m^3	1	12.81	0.0004***	m^3	1	9.86	0.002**

Apidae

Effect	Df	F	p	Effect	Df	F	p
m	1	22.06	<0.0001***	m	1	30.08	<0.0001***
m^2	1	30.43	<0.0001***	m^2	1	39.23	<0.0001***
m^3	1	34.05	<0.0001***	m^3	1	41.96	<0.0001***

Colletidae

Effect	Df	F	p	Effect	Df	F	p
m	1	56.88	<0.0001***	m	1	60.75	<0.0001***
m^2	1	58.93	<0.0001***	m^2	1	60.11	<0.0001***

Halictidae

Effect	Df	F	p	Effect	Df	F	p
m	1	18.50	<0.0001***	m	1	20.31	<0.0001***

m^2	1	23.15	<0.0001***	m^2	1	25.57	<0.0001***
Megachilidae							
Effect	Df	F	p	Effect	Df	F	p
m	1	27.56	<0.0001***	m	1	21.91	<0.0001***
m^2	1	17.25	<0.0001***	m^2	1	13.51	0.0003***

Legend of significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Apidae, Colletidae and Megachilidae show a minimum ITD in summer (from July to September), and higher values both in spring and autumn (see figure 3.7). Andrenidae show an opposite trend, Halictidae show an almost constant trend with maximum values in May-June.

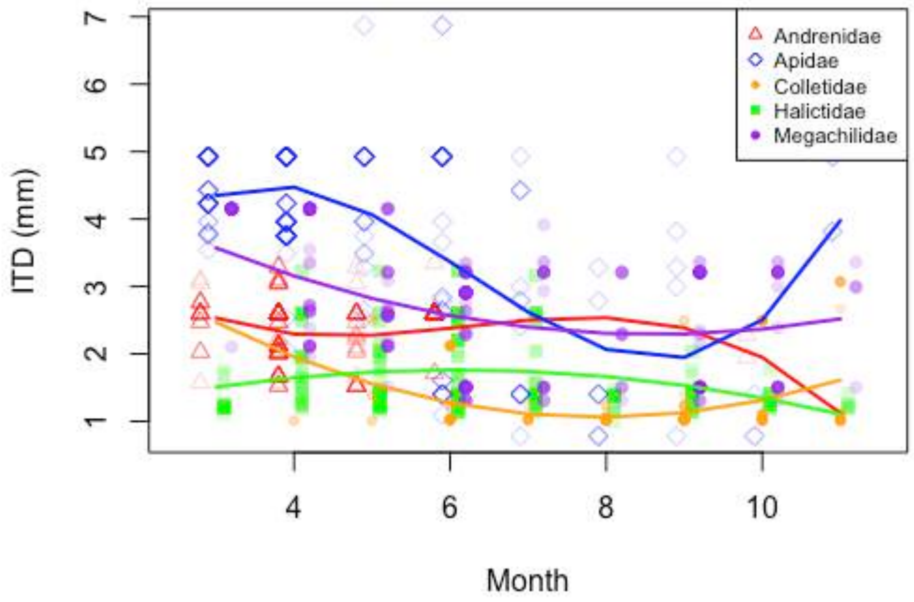

Figure 3.7: plot of ITD and time (month of collection), with model prediction.

As expected, all families show an opposite trend for S/V (see figure 3.8).

Hence, for Apidae, Colletidae and Megachilidae, we observe that, on average, bigger species with lower S/V values are dominant in spring and autumn. Andrenidae and Halictidae show different trends.

Excluding *Ceratina parvula* and *Xylocopa violacea* (upper outliers for S/V and ITD among Apidae) led to similar results for Apidae.

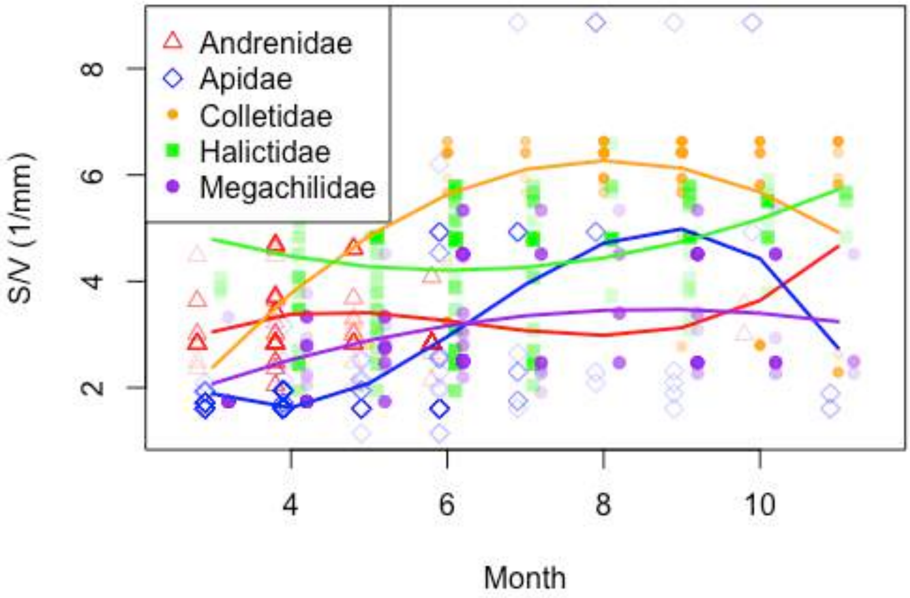

Figure 3.8: plot of S/V and time (expressed as month of collection), with model prediction.

3.7. Test of Bergmann's hypothesis: comparative method

The results of linear mixed-effects models describing the relationship between species average ITD (or S/V) and flight temperature (as observed minimum temperature T_{min} and average temperature T_{mean}) are reported in table 3.9.

Table 3.9: ANOVA results for ITD and S/V relationship with species flight temperature. T_{min} = minimum observed temperature (°C); T_{mean} = average flight temperature; f = family; Df = degrees of freedom; F = F-test value; p = p-value; R^2 = multiple R^2

log (ITD) ~ log (T_{min})*f (R^2 = 0.007)				S/V~ T_{min}*f (R^2 = 0.29)			
Effect	Df	F	p	Effect	Df	F	p
T	1	1.26	0.26	T	1	0.50	0.48
f	4	15.73	<0.0001***	f	4	9.72	<0.0001***
T:f	4	1.31	0.27	T:f	4	1.32	0.27
log (ITD) ~ log (T_{mean})*f (R^2 = 0.03)				**S/V~ T_{mean}*f (R^2 = 0.33)**			
Effect	Df	F	p	Effect	Df	F	p
T	1	9.34	0.003**	T	1	7.56	0.0070**
f	4	15.60	<0.0001***	f	4	9.51	<0.0001***
T:f	4	2.50	0.047*	T:f	4	2.26	0.067 .

Legend of significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

We observe that, using T_{min}, the effect of temperature is not significant for both ITD and S/V, while we do observe a significant family fixed effect. Using T_{mean}, we highlighted a significant effect of temperature on ITD and S/V. The family fixed effect is also significant (except for the interaction between family and temperature for S/V, meaning that families do differ in the intercept, but not in the regression coefficient). Analyses of the relationship between the response variables and T_{mean} were repeated separately for each family (see table 3.10).
We observe that most relationships are not significant. There is one exception for ITD in Colletidae, which show a negative relationship between ITD and T_{mean}.

The estimates of Pagel's λ index of phylogenetic signal for ITD, S/V, T_{min} and T_{mean} are reported in table 3.11. We observe that S/V shows a value of λ that is close to 1, meaning that phylogeny has an important influence on S/V variation. For ITD, λ is higher than 1, meaning that the ITD values in the community are more similar than expected under a Brownian motion; usually in this case λ is restricted to 1, the highest theoretical value (Freckleton et al., 2002; Münkemüller et al., 2012). A value of λ higher than 1 can also be interpreted as a higher evolutionary rate at the root of the tree (Freckleton et al., 2002). T_{mean} shows a lower level of λ, but it highlights a significant influence of phylogeny on the trait variation. For T_{min}, λ is not significantly different from 0.

Table 3.10: regression coefficients and ANOVA results for ITD and S/V relationship with species flight temperature (for each family). m = regression coefficient; sd_m = standard deviation of regression coefficient; T_{mean} = average flight temperature; f = family (And= Andrenidae; Api=Apidae; Col=Colletidae; Hal=Halictidae; Meg= Megachilidae); Df = degrees of freedom; F = F-test value; p = p-value; R^2 = multiple R^2

	log (ITD) ~ log (T_{mean})						S/V~ T_{mean}					
f	m	sd_m	Df	F	p	R^2	m	sd_m	Df	F	p	R^2
And	0.1	0.3	1	0.21	0.65	0.01	-0.04	0.05	1	0.62	0.44	0.03
Api	-0.8	0.4	1	4.06	0.056	0.16	0.12	0.07	1	3.38	0.079.	0.13
Col	-1.8	0.8	1	5.48	0.041*	0.35	0.3	0.1	1	4.60	0.058.	0.31
Hal	0.1	0.3	1	0.070	0.79	0.002	0.00	0.05	1	0.0053	0.94	0.0002
Meg	-0.2	0.3	1	0.41	0.53	0.02	0.03	0.04	1	0.56	0.46	0.02

Legend of significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3.11: Pagel's λ estimates with standard deviation for four traits

Trait	Pagel's λ
ITD	1.015±0.001
S/V	0.925±0.001
T_{min}	0.03±0.49
T_{mean}	0.460±0.001

The pGLS analyses did not yield significant relationships for any combination of response variable, effect variable and model of evolution (see table 3.12).

Table 3.12: Results of the pGLS analyses on ITD and S/V relationship with temperature. T_{min} = minimum observed temperature; T_{mean} = average temperature; BM = Brownian motion; OU = Ornstein-Uhlenbeck; Df = degrees of freedom; F = F-test value; p = p-value

		ITD model			S/V model		
Model	Effect	Df	F	p	Df	F	p
BM	log (T_{min})	1	0.92	0.34	1	0.32	0.57
	log (T_{mean})	1	1.65	0.20	1	1.44	0.23
OU	log (T_{min})	1	0.83	0.36	1	0.25	0.61
	log (T_{mean})	1	1.70	0.19	1	1.54	0.22

3.8. Test of mechanistic hypotheses: community approach

Mixed-effect linear models yielded different results for different families. The results for ITD and S/V models are similar when we consider the effect of temperature. The significance of the biomass-effect is more variable between the two approaches (see table 3.13).

Table 3.13: regression coefficients and ANOVA results for ITD and S/V temperature and total biomass (for each family). T = flight temeperature; B_m = total biomass; f = family; m = regression coefficient; sd_m = standard deviation of m; Df = degrees of freedom; F = F-test value; p = p-value

ITD ~ scale(T)+scale(B_m) + random=~1|Site										
	T					B_m				
f	m	sd_m	Df	F	p	m	sd_m	Df	F	p
And	0.02	0.03	1	0.89	0.35	0.04	0.02	1	2.71	0.10
Api	-0.62	0.08	1	58.47	<0.0001***	0.11	0.08	1	1.81	0.18
Col	-0.11	0.02	1	30.67	<0.0001***	0.04	0.02	1	3.59	0.06
Hal	0.09	0.03	1	12.43	0.0005***	0.14	0.03	1	32.44	<0.0001***
Meg	-0.28	0.05	1	30.59	<0.0001***	0.05	0.05	1	1.30	0.26

S/V ~ scale(T)+scale(B_m) + random=~1|Site										
	T					B_m				
f	m	sd_m	Df	F	p	m	sd_m	Df	F	p
And	-0.03	0.04	1	0.72	0.40	-0.05	0.03	1	54.68	0.17
Api	0.7	0.1	1	7.39	<0.0001***	-0.2	0.1	1	5.03	0.03*
Col	0.27	0.05	1	28.24	<0.0001***	-0.14	0.05	1	9.05	0.003**
Hal	-0.21	0.06	1	13.42	0.0003***	-0.33	0.06	1	35.82	<0.0001***
Meg	0.34	0.06	1	32.88	<0.0001***	-0.04	0.05	1	0.55	0.46

We observe that there is a significant effect of temperature on both ITD and S/V, except for Andrenidae. For ITD, Apidae, Colletidae and Megachilidae show a negative effect of temperature of ITD: this means that largest species are more frequent with low temperatures. For S/V and for the same three families, the trend is positive, meaning that species with higher S/V are more frequent with high temperatures. Halictidae show an opposite trend in both cases, while Andrenidae show no significant effect.

There is a significant positive biomass effect on ITD only for Halictidae, meaning that larger species are more abundant when the community biomass is higher. On the other side, all families except Andrenidae and Megachilidae show a significant negative effect of B_m on S/V. This means that in these families, species with higher S/V are more frequent when the total community biomass (proxy of resource abundance in our analysis) is lower. When both effects (biomass and temperature) are significant (for ITD, in the case of Halictidae; for S/V in the case of Apidae, Colletidae, Halictidae) we observe that the effects have a similar order of magnitude. These effects are comparable, as we performed the mixed-effects models with scaled effect variables.

3.9. Hair density effect

The results of linear OLS models describing the relationship between the residuals of the S/V model and hair density (H) are reported in table 3.14. We excluded Andrenidae from the analyses, as there was no significant effect of temperature on S/V in this group (see 3.7). For a plot of residuals and hair density see figure 3.9.

Table 3.14: OLS models coefficients and ANOVA results for $Res(S/V \sim T+B_m) \sim H$.
m = regression coefficient; sd_m = standard deviation of the regression coefficient; Df = degrees of freedom; F = F-test value; p = p-value

Family	m	sd_m	Df	F	p
Apidae	-0.045	0.006	1	53.78	4.2e-12***
Colletidae	-0.081	0.004	1	346.63	<0.0001***
Halictidae	0.007	0.004	1	3.56	0.060.
Megachilidae	-0.063	0.006	1	125.53	<0.0001***

Legend of significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

We observe that the relationship is significant and negative for Apidae, Colletidae and Megachilidae. This means that, in these groups, bees that have a higher hair density tend to have lower S/V than the average bee (and then, they are in general larger than the average bee) at the same environmental condition.

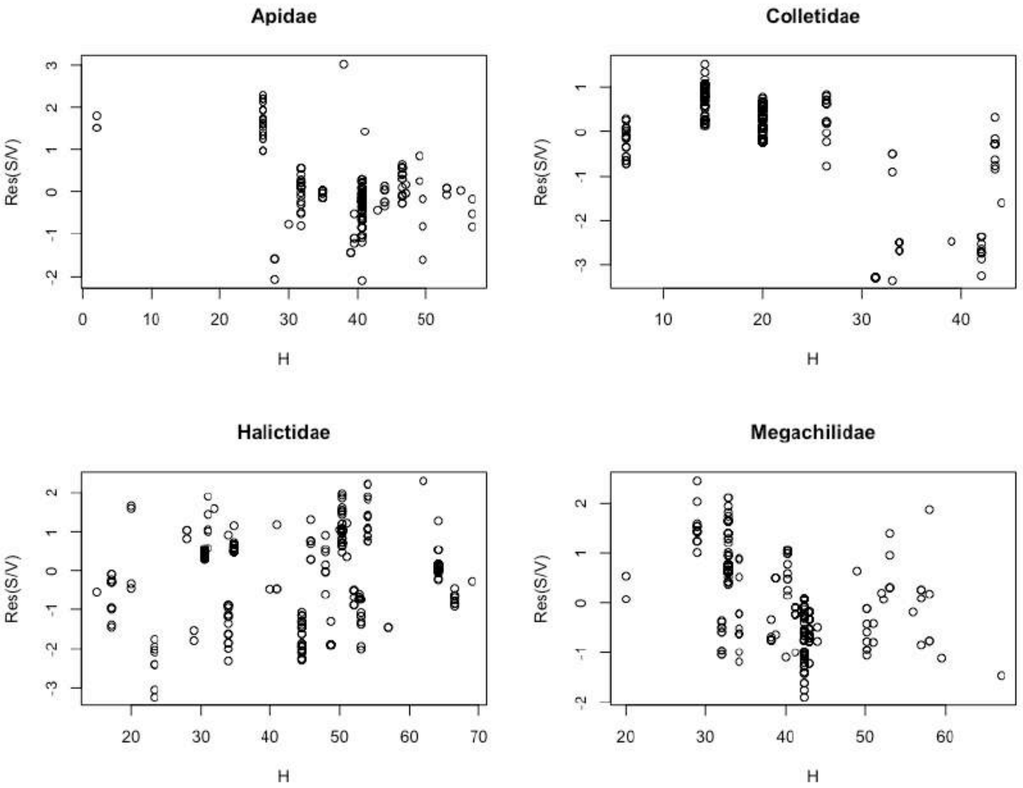

Figure 3.9: plot of residuals of S/V~T+B_m (Res(S/V)) versus hair density (H) for 4 families.

3.10. Tongue length variation

The variation of the distribution of tongue length (proboscis length, PL) along the season is represented in a violin plot (figure 3.10a and 3.10b including or excluding *A. mellifera*).

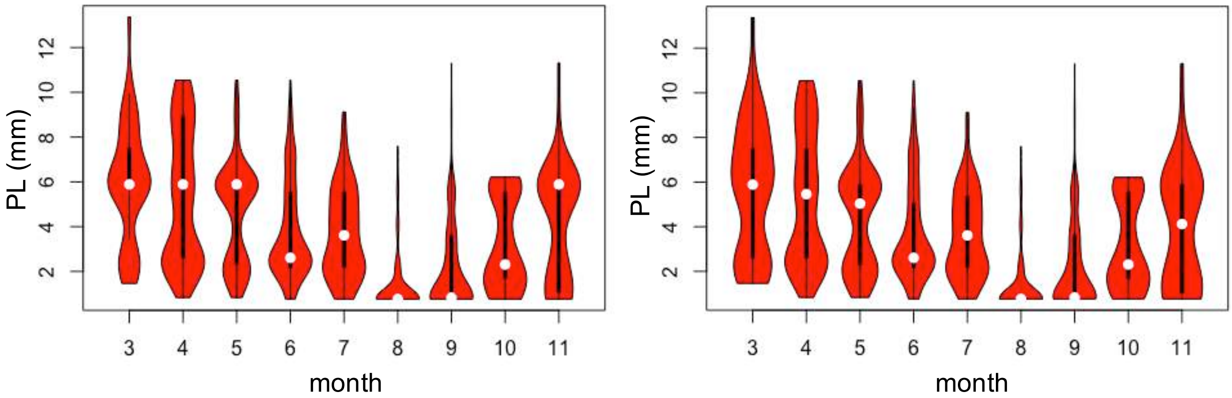

Figure 3.10: Violin plot of the distribution of proboscis length (PL) in the community along the season (a) including or (b) excluding *A. mellifera*.

We observe that the proboscis length has a similar trend than the one observed for ITD (see 3.6): we found that, at the beginning of the season, there is a consistent group of long-tongued bees (PL > 8 mm) that is greatly reduced from May to October. A group with shorter tongues (4 to 8 mm) is almost absent in August and September, when short-tongued bees (PL < 4 mm) cover most of the community. Short-tongued bees are present almost all along the season, so that, in early spring and late autumn proboscises are almost evenly distributed along the range of the variation (between 2 and 10 mm).

The results of ANOVA on the fixed-effects model describing the relationship between ITD and proboscis length (PL) are reported in table 3.15.

Table 3.15: ANOVA results for log(PL)~log(ITD)*Family.
f = family; Df = degrees of freedom; F = F-test value; p = p-value

Effect	Df	F	p
log (ITD)	1	1108.95	<2e-16***
f	4	104.22	<2e-16***
log (ITD):f	4	2.60	0.042*

Legend of significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

We observe that there is a significant effect of ITD and family (and their interaction) on PL.

The specific means of PL and ITD are plotted in figure 3.11, in a logarithmic scale. There is an overall significant positive relationship between proboscis length and ITD in the community.

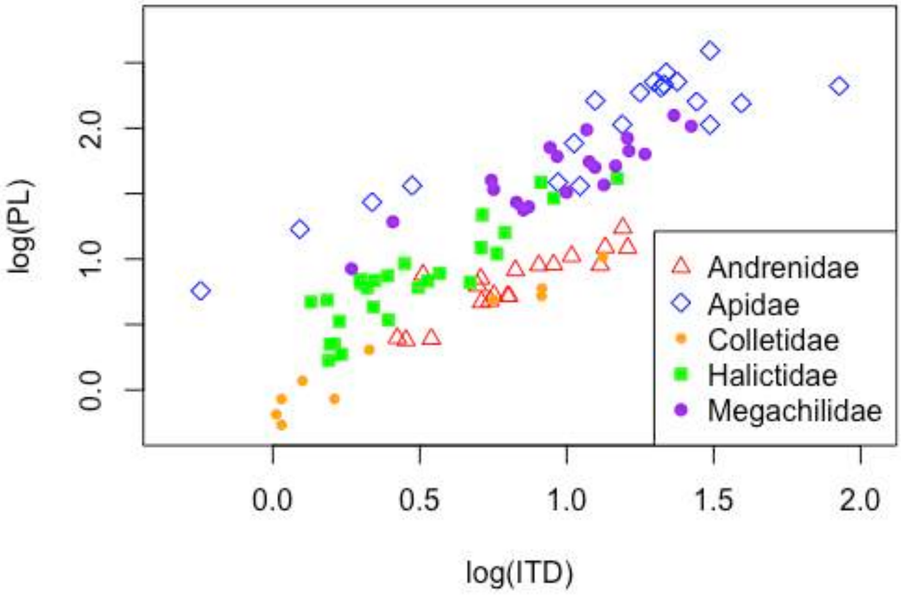

Figure 3.11: Plot of proboscis length and size (log(PL) and log(ITD)).

By performing models that described the seasonal trend of the “proboscis community” we found that the best fitting model is a constant model for Andrenidae, a 3rd-degree function of time for Apidae and a 2nd-degree function of time for the other families (see table 3.16). The data about PL along the season are plotted in figure 3.12.

Table 3.16: marginal ANOVA results for PL seasonal variation

m = time in months; Df = degrees of freedom; F = F-test value; p = p-value

Andrenidae: PL~m+random=~1|Site			
Effect	Df	F	p
m	1	1.15	0.28
Apidae: PL~m+m^2+m^3+ random=~1|Site			
Effect	Df	F	p
m	1	19.61	<0.0001***
m^2	1	32.26	<0.0001***
m^3	1	40.00	<0.0001***
Colletidae: PL~m+m^2+ random=~1|Site			
Effect	Df	F	p
m	1	67.63	<0.0001***
m^2	1	68.25	<0.0001***
Halictidae: PL~m+m^2+ random=~1|Site			
Effect	Df	F	p
m	1	20.80	<0.0001***
m^2	1	24.59	<0.0001***
Megachilidae: PL~m+m^2+ random=~1|Site			
Effect	Df	F	p
m	1	26.81	<0.0001***
m^2	1	13.79	0.0002***

Legend of significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Except for Andrenidae, which seem to show a constant trend, all other families have similar seasonal trends in PL than the ones observed for ITD (see 3.6).

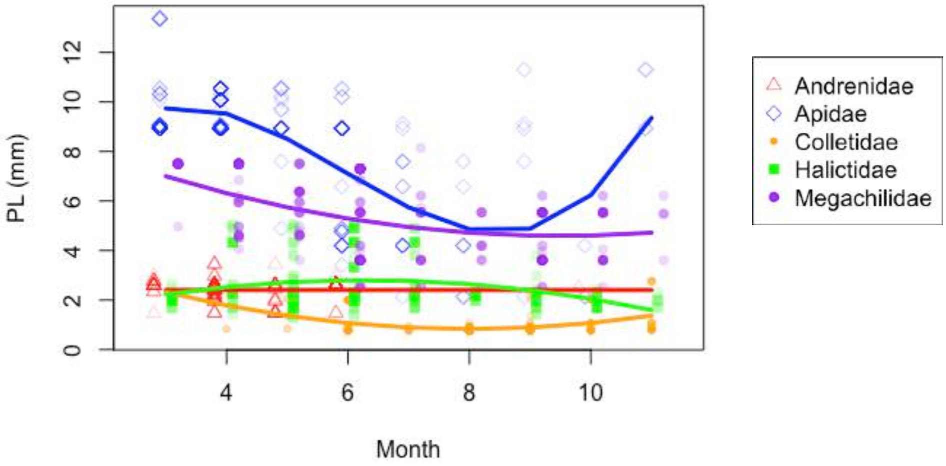

Figure 3.12: seasonal trends of proboscis length (PL) for different families (with prediction).

4. DISCUSSION

4.1. Seasonal diversity patterns

The survey showed that the wild bee community of Southern Corsica is, as expected, highly specious: we observed about 170 species from 6 families (Melittidae were collected outside the protocol). Our sample included 121 species from 5 families. We performed simple analyses on the diversity in our sample, to highlight possible effects of diversity variation on further analyses.

We observe that there is a significant change in the composition of the community along the season. Different families show different flight seasons. Apidae, Halictidae and Megachilidae are active from March to November, while Colletidae are extremely rare in the first part of the season, and become the dominant group from August to November. Andrenidae are absent from July to September. We can infer that phenology is considerably influenced by phylogenetic relationships, since bee species belonging to the same group tend to be observed in the same periods. This is also true at a finer scale, as species belonging to the same subfamily, tribe or genus can easily have similar phenologies (*e.g.* all *Ceratina* and *Xylocopa* species, both belonging to Xylocopinae subfamily, share an important part of their flight period, from June to August; *Heriades* species are almost all present from May to October).

This result is confirmed also by the phylogenetic diversity index SES_{MPD} (non-weighted). This index has always negative values, with significantly negative values in June, July, September and November. Negative values of SES_{MPD} are usually interpreted as a signal of tree-wide patterns of clustering, meaning that, all along the year, the species of the community that are present at a given moment tend to be phylogenetically closer than expected (Webb et al., 2002; Kembel, 2009; Kembel, 2010).

For both weighted or non-weighted SES_{MPD}, it is important to consider that the phylogeny we used, derived from a phylogeny of bee genera (Hedtke et al., 2013), does not include accurate information on the structure of relationships at the species level, since all species belonging to each genus are in a politomy. This part of the structure is ignored by the analyses, so that the inference we make is valuable when we refer to supraspecific taxa, and not for species. Nonetheless, if SES_{MPD} is significantly negative though ignoring species level phylogeny, it could be inferred that significant patterns of clustering are present in the phylogeny at the genus or family level.

We accounted for the specific abundances calculating Simpson's D and abundance-weighted SES_{MPD} indexes. Simpson's D describes the probability of drawing two individuals from the community, both belonging to a single species (Simpson, 1949). This probability shows important variation along the season, with more evenly distributed communities in March, May and July, and much less diverse communities in August and September. The case of August is particularly interesting, as D is higher than 0.5. This is mainly due to the presence of a single species (*Hylaeus clypearis*), which covers about the 70% of the community abundance of the month. In general, high values of D are due to either extremely low species richness or to the presence of few abundant species and many very rare species (Simpson, 1949; McIntosh, 1967). This is almost always the case for our community in a given month, since there is always a considerable proportion of species that are represented by 1 or few specimens. This tendency is particularly pronounced in late summer.

Abundance-weighted MPD describes the deviation of mean phylogenetic distance between two individuals that are randomly drawn from the community, and SES_{MPD} compares the observed MPD with a population of randomly generated pseudo-communities (Webb et al., 2002; Kembel, 2009; Kembel, 2010). We observe negative values of SES_{MPD} in May and from July to November (significantly negative in July, August and November). SES_{MPD} is positive in March and June. Negative values are expected if the community is mainly composed of closely related species (or genera) or of few abundant species and many rare species (Kembel, 2009). For instance, this seems to be the case for the month of August. At the same time, positive values are expected if the community is composed of many distantly related species with similar abundances (Kembel, 2009). This could be the case of the month of June.

These analyses show that bee species phenologies and abundances are influenced by the phylogeny. Therefore, when we make community wide analyses we must consider that at a given moment, bees in the community tend to be more closely related to the bee species that are flying in the same period, than they are to bees flying in other seasons, directly influencing morphological variation. If we observe a seasonal cline, this could depend just on the fact that the species flying at any moment are so related that they also share similar traits. Hence, we need to take into account this influence, and this could be done with two simple methods: first, performing analyses on subsamples corresponding to supraspecific taxonomic groups, as families, and second, performing analyses accounting for phylogeny.

4.2. Size and shape variation

Wild bee communities show important variation in size and body mass (*e.g.* Osorio-Canadas et al., 2016), and this is the case also for the bee community of Bonifacio. This variation is important at the community level (*e.g.* the largest bee we observed, *Xylocopa violacea*, is 183 times heavier and 8.8 times larger in ITD than the smallest, *Ceratina parvula*), but also at lower taxonomic levels, as genus (the largest species in *Andrena* genus has an ITD 2.2 times larger than the one of the smallest) or family/subfamily (e.g. both *Ceratina* and *Xylocopa* belong to Apidae: Xylocopinae). It is easy to observe that variation in shape can be important too: the PCA and MANOVAs showed that, in our study sample, this variation in size and shape was significant at an interspecific level. Interspecific morphological variation proved to be significantly larger than the intraspecific one, and the result is the same when size is not considered. This means that our measurements are able to describe morphological interspecific variation. This variation is probably mostly due to size related variation (PC1 describes more than 90% of variation) (Jolicœur and Mosimann, 1960), but there is a significant variation in shape. If we observe the results of PCA we see that all families share, with few exceptions, a similar range of variation on PC1 (then, a similar range of size variation). The PCA shows a certain degree of clustering along PC2, probably meaning that species are more similar in shape inside families.

An important variation in shape suggests that the surface-to-volume ratio can be different in bees of the same size, if they are distantly related. This justifies our approach based on S/V estimates, because this means that ITD could be not be a good proxy of S/V, and hence that we need to test if the relationship between ITD and S/V is strong.

4.3. Allometric trends

We analysed the allometric trends between morphological traits and ITD (used as a standard body size proxy for bees) (Cane, 1987; Kendall et al., 2018). This is equivalent to test if variation in shape has some relationship with variation in size. We observe that this is the case for many traits in different groups.

First, except for Halictidae, head traits show a general negative allometry with ITD. This means that larger species tend to have smaller heads, relative to ITD, than the smaller species, which is equivalent to say that smaller species tend to have relatively larger heads. This could be interpreted as a constraint on the miniaturization of head structures: sensory organs, mouth parts and central nervous system could need to stay above a size threshold in order to maintain these structures functional. This phenomenon is known to happen, at least for the nervous system, in many animal taxa, including many arthropods, insects and hymenopterans (Eberhard and Wcislo, 2011; Quesada et al., 2011; Seid et al., 2011; Niven and Farris, 2012; Polilov, 2015). A similar allometry was observed for mouth parts in bees (Cariveau et al., 2016). The phenomenon is so generalised for the central nervous system that this negative allometry is also called Haller's rule (Seid et al., 2011). To our knowledge, there is no information on this allometry in bee nervous system, but we suggest that similar constraints could lead to the patterns we observe.

We observe an isometric trend in the traits of the thorax and abdomen in Andrenidae and Halictidae. The thorax is flatter and shorter in larger Colletidae species, it is relatively flatter in larger Apidae and Megachilidae. The abdomen is wider in larger Apidae, while it is stouter in larger Colletidae and Megachilidae.

These trends in body shape, though complex and variable according to different phyletic groups, certainly have influence on the surface-to-volume ratio:

according to the family, bigger species could have a different S/V than the one predicted by isometric growth. Hence, a part of the variation in S/V in the community is likely to be determined by allometric trends between ITD and other morphological traits. Nonetheless, we found no general trend suggesting that bigger species, which geometrically tend to have lower S/V ratios, also tend to have a shape that further reduces their S/V, for instance, without stouter forms. Neither is this observed in smaller species.

4.4. Relationship between body mass, volume, ITD and S/V

We observed a strong positive relationship between ITD and body mass. This confirms previous results (e.g. Cane, 1987; Kendall et al., 2018), corroborating the fact that ITD is a good proxy for body mass in bees. There is a significant effect of family (and then, phylogeny) on this relationship.

We obtained a strong positive relationship between volume and mass. This result has two consequences: the first is that our estimation of body volume is reliable, because variation in volume is proportional to variation in body mass; second, body density seems almost constant among different species (at least among species of the same family, since a family effect is significant). To our knowledge, no estimation of body volume has ever been used in a study on bees. The rough description of body shape we provided, from which a consistent estimate of body volume and body surface can be computed, enabled a thorough test of the physical hypothesis behind Bergmann's rule.

Finally, ITD also proved to be a very good predictor of S/V ratio ($R^2 \approx$ 98.6%). Hence, though, at a given size, a different shape must have some influence on S/V, at the community level the S/V variation is very well explained by variation in size. This result also validates the approach used in previous studies on Bergmann's rule, where ITD is used as a proxy of S/V (*e.g.* Osorio-Canadas et al., 2016; Gérard et al., 2018).

4.5. Seasonal trends of size and shape

When we analysed the trend of bee body size distribution along the season, we observed that there is an overall reduction of body size in the community from March to August, with the largest and the average-sized bees gradually disappearing, while the small bees become predominant. The trend is partially inverted in the last three months of the survey.

Mixed-effect model analyses showed that the relationship between size (or S/V) and the third power of time (expressed in months) is significant. A family effect and a collection-site effect are also significant. When we performed a model selection on family subsamples, we found that the model that fitted best both size and S/V relationship with time was a third-degree function of time expressed in months in the case of Andrenidae and Apidae, while it is a function of the second degree for Colletidae, Halictidae and Megachilidae. The similarity of results between size and surface-to-volume ratio is expected, because of the strong relationship we observed between the two.

Environmental temperature being lower in spring and autumn than in summer, we expect smaller sizes in summer, if the rule is valid along this temporal gradient (Osorio-Canadas et al., 2016). For three families, Apidae, Colletidae and Megachilidae, we observed that size reaches minimum values in summer or late summer (July-September), while higher values are observed in the first and the last parts of the season. In any case, values observed in autumn (October-November) seem not to reach the values observed in spring (see figure 3.7). Surface-to-volume ratio shows, as expected, an opposite trend, with maximum values in late summer. Lower values are observed in spring and autumn, with minimum S/V in spring. Hence, these three families follow a seasonal Bergmann's cline, *i.e.*, they show larger body size and lower surface-to-volume ratio in colder months (Bergmann, 1847; Osorio-Canadas et al., 2016).

Andrenidae and Halictidae do not show a seasonal Bergmann's cline: Andrenidae show a maximum size in late summer, and Halictidae show an almost constant trend with maximum size in June. Opposite trends were found for S/V.

It must be considered that these models describe the trend of the abundance-weighted mean of our size estimate (or S/V estimate) in each family along the season. Hence, our results state that, in summer, the subsamples of the community corresponding to Apidae, Colletidae and Megachilidae, show an overall majority of relatively small bees with high surface-to-volume ratio, and that opposite situation is observed in spring and autumn. This does not necessarily mean that the largest species we observed were collected only in spring and autumn, but that in summer, large species are less abundant than small species, and vice versa for colder periods.

Nonetheless, the observed pattern is consistent, for at least three families, with both the hypotheses we provided.

Bergmann's thermoregulation hypothesis predicts an optimisation of surface-to-volume ratio in order to reduce heat dispersion in colder climates (Bergmann, 1847; Blackburn et al., 1999; Watt et al., 2010; Osorio-Canadas et al., 2016).

At the same time, the resource-abundance hypothesis suggests that we expect bigger sizes (and then, smaller S/V) with more important resource availability (Atkinson and Sibly, 1997; Watt et al., 2010). We do not have precise information about flowering, pollen and nectar abundance and quality along the year for our community. We used the total monthly collected biomass as a proxy of the floral resources wild bees feed on: this approach is based on some assumptions that are discussed later (see 4.6.2). The fact that our biomass estimate reflects the food availability is supported by the similarity of the seasonal trend we observe in bee biomass, with the trend of flowering activity and nectar sugar content observed in a similar habitat (Flo et al., 2018). Since our estimate of resource abundance is more important in spring, we expect larger size (and smaller S/V) in this period.

Our resource estimate is low at the end of the season, and this could explain why body size at the end of the season does not reach the same values as in spring.

The observed pattern is thus consistent with both the mechanistic hypotheses we considered (at least for some phyletic groups), calling for further tests for these hypotheses.

4.6. Test of mechanistic hypotheses

4.6.1. Bergmann's thermoregulation hypothesis

To test for the validity of Bergmann's hypothesis, at first, we performed a classical comparative analysis, testing the relationship between the species values of ITD (or S/V) and a species-specific flight temperature estimate (the minimum observed temperature or the average observed temperature of the species). The minimum observed flight temperature of a species is considered the most informative parameter when it is necessary to evaluate bee thermal ecology (Osorio-Canadas et al., 2016). In fact, even endothermic bee species show a minimum environmental temperature below which they are not able to maintain thoracic temperature in a physiological range for flight (Heinrich, 1993; Woods et al., 2005; Peters et al., 2016). Higher temperatures seem to be less limiting, as many bee species are able to reduce body temperature with both physiological mechanisms (*e.g.* transferring heat from the thorax to the head, abdomen and legs, in *A. mellifera*) (Heinrich, 1980; Heinrich, 1993; Peat et al., 2005a; Osorio-Canadas et al., 2016), or behaviour (such as flying in colder parts of the day or increasing flight speed) (Heinrich, 1993; Willmer and Stone, 2004).

When we observe a specimen of a species, this automatically means that the maximum environmental temperature of the day in which it was observed was higher than the threshold for the species to fly, while this is not automatically true for the minimum or average daily temperature. Nonetheless, measuring this minimum of maximum daily temperature can be hard, especially for rare species. The delay of the first observation of a species with its first flight can be variable and the gap between the observed minimum flight temperature and the real one can vary importantly from species to species, from period to period. Therefore, we also introduced a measure of average flight temperature.

Fixed-effects models were not significant when the minimum temperature was the effect variable: we only observed that families significantly differed in their average size and S/V. When the average temperature was the effect variable, we found a significant effect of temperature both on size and S/V. The model for S/V had the highest proportion of explained variation ($R^2 \approx 33\%$).

When performing an accurate analysis at the family level, we observed that most relationships between size (or S/V) and the average flight temperature, are not significant, except for Colletidae for body size, for which the relationship is negative as expected: larger Colletidae species do have a lower average flight temperature, and the model explains about the 35% of the variation. The relationship is negative for ITD also in the other two families that proved to follow a Bergmann’s cline (Apidae and Megachilidae), but the relationship is not significant. Interestingly, Andrenidae and Halictidae show a converse trend, though not significant. The other response variable, S/V, shows opposite trends for all families, but the relationships are not significant. According to this analysis, only Colletidae data significantly support the prediction provided by Bergmann’s hypothesis.

We introduced phylogenetic information in the analyses, at first by calculating Pagel’s λ phylogenetic signal estimate (Pagel, 1999). Though the phylogeny we used was not accurate at the species level, the analyses highlighted a significant influence of phylogeny on size and S/V and the average flight temperature. The case of the minimum flight temperature is quite different, as the estimate is low (0.03), but the error is so high that we cannot infer much about the influence of phylogeny on the minimum flight temperature.

Since we observed a phylogenetic signal, and we wanted to test if the relationships among these variables (size, S/V and temperature estimates) we observed held even accounting for phylogeny, we performed pGLS analyses. We introduced either a Brownian motion or an Ornstein-Uhlenbeck model of evolution, testing the effect of average (or minimum) flight temperature on size (or S/V). These analyses showed no significant effect of temperature on body size and surface-to-volume ratio, when accounting for phylogenetic relationship. This result was expected for the minimum flight temperature, since the relationship was not significant in the classical comparative analysis. For the average temperature, the relationship with ITD (or S/V) was not significant in most cases. When considering the entire phylogeny, the signal carried by a restricted subsample of the tree (*e.g.* Colletidae) could be lost. This result is different from what was observed in a previous similar study (Osorio-Canadas et al., 2016). In that case, a similar Mediterranean community was studied, and relationship between the ITD and observed minimum flight temperature were analysed accounting for an applicable phylogeny (Osorio-Canadas et al., 2016): the analyses yielded a significant negative relationship (Osorio-Canadas et al., 2016).

There are at least three other explanations that could explain why we do not observe significant clines, when similar trends have been observed in other cases (Osorio-Canadas et al., 2016): first, the tree we used was based on a tree of bee genera (Hedtke et al., 2013), with all the species of the same genus diverging from the same internal node. We suppose that information on phylogenetic relationships at the species level could have highlighted a different or more significant pattern. In fact, the differences between traits among closely related species are the most informative in phylogenetic comparative analyses (Harvey and Pagel, 1991), and the phylogenetic relationships among closely related species are poorly described by our phylogeny. Second, we introduced two different models of evolution that can be inadequate to our data. In fact, the Brownian motion model describes evolution by pure drift, with trait values changing randomly from the value of the last common ancestor of the species (Butler and King, 2004). Brownian motion is thought not to be able to describe the case of a generalised selective pressure in a group, and therefore, we introduced an Ornstein-Uhlenbeck model, which can describe also evolution under natural selection (Butler and King, 2004). Nonetheless, also the latter can be inadequate to explain our data if there is no general optimum value of the trait (body size or S/V) valid throughout the tree (Butler and King, 2004). This could be the case for our community, if in different species or groups different selective factors caused different trade-offs with thermoregulation abilities. Third, we did not take into account the possibility of a body size threshold below which bee species are not endotherms (Bishop and Armbruster, 1999; Osorio-Canadas et al., 2016). Osorio-Canadas found this threshold by performing piecewise linear regression analysis, *i.e.* fitting two different linear models below and above a moving threshold (Osorio-Canadas et al., 2016). The presence of such a threshold could influence the results we have. We did not introduce a similar analysis because we do not have any information about the real thermoregulatory abilities in our community. Comparative analyses did not yield significant results, with few exceptions.

We also used a community-wide approach accounting for specific abundances: it allows to describe the community of bees that are present at a given environmental condition and then, in our case, it allows to understand whether the community is mostly composed of bigger bees at low environmental temperatures or with more abundant resources.

The mixed-effect models yielded a highly significant linear relationship between size (or S/V) and temperature for four out of five families. Three families (Apidae, Colletidae, Megachilidae) have positive relationship between S/V and temperature; they show an opposite pattern for size. Halictidae show a positive relationship between size and temperature (negative between S/V and temperature). Andrenidae do not show significant relationships. Interestingly, the three families that showed a Bergmann's seasonal cline also conform to the prediction made by Bergmann's mechanistic hypothesis, *i.e.* that bees facing lower temperature have on average larger size and smaller surface-to-volume ratio (Bergmann, 1847). The other families show a converse trend or no trend. Our results on body size and S/V patterns suggest that the thermoregulatory hypothesis could be valid for three families.

The thermoregulatory (or heat conservation) hypothesis, is usually tested in bees using a proxy of body size, ITD. This, in turn, is a proxy of surface-to-volume ratio (*e.g.* Osorio-Canadas et al., 2016; Peters et al., 2016; Gérard et al., 2018). We followed this approach, so that we could compare our results with previous literature, but we also extended it to a direct estimation of S/V ratio to directly test Bergmann's mechanistic hypothesis.

This study confirms that Bergmann's clines are not generally present in all groups of bees, even for a seasonal gradient (Hawkins, 1995; Shelomi, 2012; Osorio-Canadas et al., 2016; Peters et al., 2016; Ramirez-Delgado et al., 2016; Gérard et al., 2018). Nonetheless, we show that changes in size phenology in a temperate bee community are correlated with temperature seasonal trends. Therefore, our results corroborate the hypothesis that temperature has an influence on structuring the community along seasons, and that reduced heat loss by changes in size and shape could have had an important adaptive value in the evolutionary history of bees. Moreover, we highlighted the usefulness of a direct test of mechanistic hypotheses.

The lack of generality could be explained by the influence that other factors (hair length and density, colouration or behavioural mechanisms) can have on thermoregulation (Osorio-Canadas et al., 2016; Peters et al., 2016). Darker colouration or specific behaviour, as foraging in favourable microclimates, could facilitate the reach of operating flight temperature (Herrera, 1995; Willmer and Stone, 2004; Osorio-Canadas et al., 2016; Peters et al., 2016) (the influence of hair density will be discussed later). In some cases, these mechanisms could have a higher impact than the impact of surface-to-volume ratio on thermoregulation.

Moreover, we found that the only families that do not show a Bergmann cline are Andrenidae and Halictidae. It has already been proposed that some genera belonging to these two families (*Andrena*, *Lasioglossum* and *Sphecodes*) are responsible for deviation from prediction (Osorio-Canadas et al., 2016): *Andrena* and *Lasioglossum* species are able to fly at lower operating temperature than other bees of the same size (Bishop and Armbruster, 1999; Osorio-Canadas et al., 2016). *Sphecodes* species are known to be kleptoparasitic on *Andrena* and *Lasioglossum* and could operate at lower temperatures, as they do not need to transport pollen and nectar on long distances (Stone and Willmer, 1989; Osorio-Canadas et al., 2016). This could explain the pattern we observe for these two families, even if a clear explanation of these characteristics is still needed (Osorio-Canadas et al., 2016). These families are mostly composed of ground-nesting bees. Gérard showed that both ground-nesting and stem-nesting bees show a Bergmann's latitudinal cline, but that the trend is less strong in the ground-nesting, which could be buffered against temperature variation (Gérard et al., 2018). This hypothesis needs further tests, but we suggest that finding a signal of discrepancy in Bergmann's clines related to nesting-traits could be difficult, since the nesting behaviour can be highly conserved within phyletic groups (in fact, all Andrenidae and most Halictidae species are ground-nesting) (Michener, 2007).

Finally, to test if hair density influences S/V variation, we modelled the relationship between hair density and the residuals of the mixed-effect model for S/V depending on temperature and biomass, for each family. A previous study showed that, size being equal, bees with longer thoracic hair can fly at significantly lower air temperatures (Peters et al., 2016): we wanted to test if a similar effect was carried by hair density. We hypothesised that at a given environmental condition, bees with higher hair density should have a higher than average S/V: heat loss should be mitigated by a higher hair density, allowing a higher dispersion surface. We tested this hypothesis on the four families for which a significant relationship between S/V and temperature was observed. Our results show that the relationship between the residuals of S/V and hair density is significant and negative for three families (Apidae, Colletidae and Megachilidae) meaning that bee larger than expected in groups following Bergmann's rule, also show high hair density. Hence, our prediction is not verified. We suggest that, if the selection for higher hair density is strong for bees flying in cold months, this means that bees flying, for instance, in March will have in general much denser hair than the average bee flying in summer months. Since larger bees, with low S/V, usually fly in cold months, they will tend to have negative residuals, and the same happens with small bees, that will have in general positive residuals and less hair, influencing the relationship we studied. Moreover, our analysis considered hair density but not hair length. Bees with the same hair density but different hair lengths could differ in their thermoregulatory abilities (Peters et al., 2016). We suggest that further investigation of this effect considering both hair length and density is needed. Finally, we must consider that bees often use hair, especially thoracic, to collect pollen (Michener, 2007). Larger bees (with lower S/V) obviously need to collect more pollen to survive and to feed their larvae. There may be a positive allometric relationship between size and hair density, due to the non-linear relationship between body surface and the volume of pollen needed to feed larger larvae, and this could influence our results.

4.6.2. The resource-abundance hypothesis

The effect of monthly collected biomass on body size was always positive, but significant effect was found only in the case of Halictidae. Effects on S/V was always negative, and significant for all families except Andrenidae and Megachilidae. Hence, our analyses suggest that one to three families follow the trend predicted by the resource-abundance hypothesis, *i.e.*, that food resources availability positively influences body size. Resource abundance could be higher either because of a higher amount of available food, or because of a reduced competition (Watt et al., 2010). The reason why this abundance should be positively correlated with body size is still discussed (Watt et al., 2010). Similar effects were originally observed along latitudinal clines in endotherms, especially mammals (McNab, 1971; Yom-Tov and Nix, 1986; Geist, 1987; Watt et al., 2010), but a positive effect of food income on adult body size was observed in a bee species (Johnson, 1988; Johnson, 1990). Some proposed that reaching larger size with more resources is adaptive in habitats with seasonal unpredictable resource: larger animals can grow and stock resources more rapidly than the small ones, with a positive effect on survival rates (Boyce, 1979; Watt et al., 2010).

To our knowledge, a significant statistic effect of resource on body size along a season has never been observed in a bee community, and hence no specific explanation has previously been proposed, and mechanisms proposed for latitudinal gradients are still discussed (Watt et al., 2010). Nonetheless, our analyses yielded a positive effect of resource abundance on body size even for a taxon that seems not to show a seasonal Bergmann cline (Halictidae). This suggests that correlation between resources and temperature alone is not sufficient to explain the pattern we observed between size proxies and biomass, even because the correlation proved to be not too strong (correlation index = -0.55). In any case, we believe that this hypothesis needs further investigations, possibly accounting for finer and more direct estimations of available resources for each species or group of species.

In fact, we used community biomass as a proxy of available resources: this way, we made several assumptions. We assumed that the collection method had little or no bias in collecting different species, and then, that different abundances in the collection reflected different abundances in the community. We considered this to be a valid assumption, because the chosen collecting method is considered the most efficient for bee communities (Westphal et al., 2008). Second, since we performed our measurements only on females, we assumed that the biomass associated with females was strongly correlated with total biomass. Moreover, we know that other species were present in the area, since we excluded species observed outside the protocol and species whose collected individuals were only males or not intact. We, therefore, assumed that the trend of the biomass of the species included in the study reflected the trend of community biomass. Finally, we assumed that the community biomass that we observed in a certain period reflected the abundance of available resources. This is probably the strongest assumption, as we must consider that the biomass of a single bee, when it leaves the nest, is obtained by food collected in the previous flight season or in the previous weeks or months (Michener, 2007). At the same time, bees consume resources (*i.e.* nectar and pollen) to fly, and then more bees (or larger bees) will need more available food.

The pattern we observe in biomass is consistent with the observed patterns of plant flowering activity in Mediterranean areas, with lower flowering activity and lower sugar content in nectar in summer than in spring (Flo et al., 2018). Moreover, humidity has positive relationship with both nectar volume production and nectar sugar content (Petanidou, 2007), and we know that Bonifacio has a dry hot climate in summer months. More flowers in spring mean more pollen and nectar available, and a higher sugar content in nectar means more nutrient nectar, hence, more food of higher quality available for bees. These observations support our assumption that the total biomass is correlated with resource abundance.

4.6.3. The relative importance of the two effects

Some proposed that it is difficult to separate the effect of temperature and resources on body size (Yom-Tov and Nix, 1986), as these two variables are in part correlated (and this is the case also for our data), but we highlighted different (in some cases, opposite) trends for resource abundance and temperature. We observe that, except for Andrenidae, a negative relationship between available resources and S/V is significant for all families. This could be a consequence of the geometric relationship between body size and surface or volume: we do not have a plausible mechanism that could explain an influence of resource-abundance on shape (except allometric trends with size).

Our results could suggest that the influence of resource abundance on body size could have a similar influence than Bergmann's rule, on a seasonal gradient. The effect of changes in temperature seems to have, for most families, the same magnitude of the effect of changes in resource abundance (when both trends are significant). For Andrenidae and Megachilidae the effect of biomass on body size is not significant, but we do not have plausible explanations for this observation.

4.7. Seasonal trends in proboscis length

Confirming previous studies, we observed a positive relationship between body size and proboscis length (Cariveau et al., 2016): the length of the tongue in bees is well predicted by bee body size in our community, and larger bees do have, in general, longer proboscises (Cariveau et al., 2016).

When we analysed the seasonal variation of proboscis length, we observed that this has a similar form than the one we observed for body size: except for Andrenidae, which show a constant trend, all families follow the same type of trend than the one observed for body size. Hence, for Apidae, Colletidae and *Megachilidae*, proboscises tend to be shorter in summer (August-September), and longer in the first and last parts of the season. On the opposite, Halictidae have longer proboscises in summer, but, on average, the community is composed of bees with shorter tongues in summer.

This result is particularly interesting, because both bee body size and tongue length are known to influence plant-pollinator relationship between flower plants and bees (Vivarelli et al., 2011; Cariveau et al., 2016). In particular, proboscis length can set a physical limit to the ability of a bee to forage on a flower (Borrell, 2005). A long tongue can guarantee an access to a wide variety of flowers, leading to a more generalist diet, and a generalist diet seems to be the rule in bee groups with long tongues as Euglossini (Borrell, 2005). At the same time, flowers with longer corolla tubes have less visitor species and are hence specialised for bees with long tongues (Borrell, 2005). Other studies provided interesting results: the flower handling time can depend on tongue length (Harder, 1983), and bumblebees with short tongues were more efficient, compared to bumblebees with longer tongues, in foraging on flowers with short corolla tube lengths (Plowright and Plowright, 1997). Hence, bees are thought to show a coevolutionary arms race with the plants they pollinate (Borrell, 2005). Our results corroborate the hypothesis that selection on thermoregulatory ability could have an impact on phenology and body size evolution, confirming previous results (Osorio-Canadas et al., 2016; Peters et al., 2016; Gérard et al., 2018). Since variation in body size is related to proboscis length, we can infer that environmental temperature indirectly shapes the pollinator interaction with flower plants, causing important changes in the average tongue length along the year, with consequences for both bees and plants.

4.8. Limits and perspectives

The collection we used was made to study bee biodiversity in Southern Corsica and its variation along time, since late 19th century. This means that the sampling was not explicitly meant on the purpose of studying Bergmann's rule in bees. This has some consequences: the most important is that precise temperature and weather data were not available for all specimens. Environmental temperature is a key variable in our analyses, but we did not have precise measurements of air temperature in the time and place where each specimen was collected. This could have been done collecting temperature data while performing the transect-walks, or measuring the mean daily temperature of the day of the collection for the specimens caught in pan traps (*e.g.* Peters et al., 2016). This is particularly important if we consider that the dataset we used provided data for a geographic grid with a 0.25-degrees-resolution in latitude and longitude (Haylock et al., 2008), giving the same temperature estimate for 6 out of 7 sites, because they were located in the same cell. Moreover, we are forced to use the dataset of maximum daily temperature, but this is clearly an overestimation of the flight temperature of bees, since they were obviously not all collected at the maximum daily temperature, a problem that could be avoided, at least for bees from transect walks, if precise data were available. Precise thermal activity thresholds for each species could have been measured with laboratory experiments, exposing bees to artificially modified temperature (*e.g.* Peters et al., 2016).

Moreover, the sampling was performed only in 2017. Many morphological traits (including body size) are thought to depend on environmental condition (for instance, during the development) even with non-adaptive mechanisms: for instance, temperature is thought to influence adult body size in ectotherms, with bigger body sizes at lower developmental temperature (Atkinson and Sibly, 1997; Angilletta and Dunham, 2003). A replication of the survey for some years could allow to account for the variation caused by environmental stochasticity on bee phenology and morphology.

Phylogenetic constraints could have a role in limiting adaptive size or shape evolution (Osorio-Canadas et al., 2016), and our analyses highlighted the presence of a phylogenetic signal on some traits. Nonetheless, the phylogenetic approach we used was probably affected by the quality of our phylogeny. A complete phylogeny of our community at the species level is needed, but these data are still not available and bee phylogeny remains debated (Danforth et al., 2013; Hedtke et al., 2013).

In addition, we did not consider that inside a family, lower taxa could have shown different patterns. It is known that some genera do not follow Bergmann latitudinal clines (*e.g. Bombus*) (Ramirez-Delgado et al., 2016), and this could happen also on a temporal gradient (Osorio-Canadas et al., 2016). Other authors have studied size variation at the genus level, highlighting different clines among a small group of genera (Gérard et al., 2018). In our case, many genera were represented by only one species in our community, and some families were represented mostly by one genus (*e.g.* Andrenidae) so we decided not to analyse patterns of variation among genera.

Moreover, we did not consider the presence of intraspecific variation (*e.g.* due to genetic variation or phenotypic plasticity). This approach proved to be impossible for us, given the small abundance we had for many species, but we must consider that a strong selection on thermoregulation ability could have also shaped the distribution of intraspecific size and shape variation inside species. A similar pattern, that has already been observed in other Bergmann clines in bees (*e.g.* Peat et al., 2005a; Peters et al., 2016), could influence the community pattern. Phenology, body size and shape are also influenced by other ecological interactions, such as predation, or competition (Watt et al., 2010). We suggest that this would have been possible studying a little number of abundant species with long flight periods (*e.g.* some bumblebee species).

In general, sample sizes for hair density and tongue length should be extended, to cover a higher amount of species, and data about hair length should be included in the analyses (Peters et al., 2016). Interestingly, the collection can still be exploited in this direction.

Finally, we believe that we only partially met the recommendations on this research field proposed by Shelomi (Shelomi, 2012). Further research should include larger sample size for each species, with investigation of species variation along the flight season (Shelomi, 2012): this way, it would be possible to observe intraspecific Bergmann's clines (if present) and to better describe the interspecific one, possibly integrating both variations in the analyses (Classen et al., 2017). All sexes (or castes in social species) should be considered (Peat et al., 2005a; Shelomi, 2012; Scriven et al., 2016). Factors like colouration, hair density and length, behaviour or physiology should be taken into account, as they could influence thermoregulatory abilities in bees (Osorio-Canadas et al., 2016; Peters et al., 2016). By now, many of these traits are poorly studied for many species of our community.

4.9. Final considerations

In conclusion, our study suggests that three out of five families respect the prediction made under Bergmann's thermoregulation hypothesis. Though confirming previous results (Shmida and Dukas, 1990; Osorio-Canadas et al., 2016), we provide a direct test of the hypothesis, focusing on the mechanism rather than the pattern. This test was made possible by a simple description of bee body shape and size, extending the previous approaches based on proxies.

The validity of Bergmann's rule along seasons has several important consequences on the ecology and evolution of bees. Bees are the most important pollinators for crops and wild plants (Peters et al., 2016), and for instance, body size or the length of the tongue proved to influence the ability of a bee too forage on different species according to the size of their flowers, and the length of the tongue proved to be related to body size (Harder, 1985; Shmida and Dukas, 1990; Peat et al. 2005b; Cariveau et al., 2016). Moreover, bee phenology is certainly influenced by plant phenology (and vice versa) (Borrell, 2005), but the optimal size to forage on a certain species could be different from the optimal size to reduce heat loss. This means that several contrasting selective factors (thermoregulation, foraging, but also resource abundance, predation…) could act on body size at the same time, and that the variation we observe is probably the result of a trade-off (Gérard et al., 2018). We suggest that this trade-off should lead to larger deviation from Bergmann's rule for the rare specialist bee species, whose phenology is strictly tied to the phenology of a few plant species (Willmer and Stone, 2004).

At the same time, bee phenology, size and shape obviously influence the evolution of flowering activity, phenology and flower size and shape in flower plants (Borrell, 2005; Vivarelli et al., 2011). Interestingly, our results suggest a possible relationship between flower resources and bee body size, that is yet to be investigated. The influence of selection on thermoregulation abilities in this coevolution pattern is still unclear, but it could be important.

Finally, in a context of global warming, changes in environmental temperature are expected to induce phenological shifts and changes in size in species, if Bergmann's seasonal rule is valid (Scaven and Rafferty, 2013; Osorio-Canadas et al., 2016). Selection on thermoregulation would be reduced by increasing temperature, and then we expect a general reduction in body size (Scaven and Rafferty, 2013), which could influence the plant-pollinator interaction (Vivarelli et al., 2011). A study conducted on 18 northern European bee species showed a significant shrinkage of females of large bee species in the last 150 years, (Oliveira et al., 2016), but the authors suggested that the cause were a reduced quality of the habitats and resource abundances. We believe that further studies on Bonifacio's community, for which two collections of bees, distant in time, are available, could be useful to test this hypothesis.

5. CONCLUSION

Our study focused the seasonal morphological variation in a wild bee community, and on the extension of Bergmann's rule on a seasonal temperature gradient. On one hand, our approach, was, similarly to previous studies, based on proxies of body size (*e.g.* Gérard et al., 2018). On the other hand, we developed a novel approach, that required several morphometric measurements, to obtain a simple description of bee body shape. These measurements were used to calculate a geometric parameter of bee bodies, the surface-to-volume ratio.

Both approaches led to similar results, showing that some bee families followed a seasonal Bergmann's rule: we observed bigger bees, with lower surface-to-volume ratio, in colder months. This confirmed previous results, that showed that *sensu lato* Bergmann's clines are not a general phenomenon in bees (Shelomi, 2012; Osorio-Canadas et al., 2016; Peters et al., 2016; Gérard et al., 2018). Nonetheless, our study extended previous research with a direct test of the effect of temperature on surface-to-volume ratio.

We showed that, in some bee families, there is a significant relationship between body size (and surface-to-volume ratio) and two environmental factors: environmental temperature and resource abundance.

For environmental temperature, we observed a negative relationship with body size (and positive for surface-to-volume ratio) for *Apidae*, *Colletidae* and *Megachilidae*. Hence, these families respected the prediction made by Bergmann: species that are subject to lower temperatures should be selected to reduce their thermoregulation effort, increasing body size and reducing surface-to-volume ratio (Bergmann, 1847).

Moreover, we observed a positive relationship between body size and the community biomass, which we assumed to be a good proxy of resource abundance, for three families: *Apidae*, *Colletidae* and *Halictidae*. This followed the prediction made by the resource-abundance hypothesis, the explanations of which are still discussed (Watt et al., 2010) and lacking for insects.

In conclusion, our results suggest that selection on thermoregulation abilities, among other factors, could have influenced body size, shape and phenology evolution in wild bees.

Since we observed a variation in size along the season that is similar to variation in tongue length, we suggest that this influence could have shaped not only bees' life histories, but also their interaction with the flower plants they pollinate, with evolutionary consequences on both plants and bees that are yet to be understood (Borrell, 2005; Vivarelli et al., 2011).

Finally, our result suggest that global warming could have an influence on bee body size and shape evolution (Scaven and Rafferty, 2013), with unknown consequences for flower plants.

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APPENDICES

Table A1: species list and sample sizes (N), sample size for hair density (H) and availability of PL data (T, Y=available, N = not available). And = Andrenidae; Api= Apidae; Col= Colletidae; Meg=Megachilidae

Family	Species	N(H)T	Family	Species	N(H)T	Family	Species	N(H)T
And	Andrena alfkenella	4(3) Y	Api	Tetraloniella fulvescens	1(1) Y	Hal	Lasioglossum pygmaeum	2(1) Y
And	Andrena cinerea	5(5) Y	Api	Thyreus affinis	1(1) N	Hal	Lasioglossum soror	1(1) N
And	Andrena fabrella	5(5) Y	Api	Thyreus histrionicus / picaron	4(4) Y	Hal	Lasioglossum transitorium	3(3) Y
And	Andrena flavipes	5(5) Y	Api	Xylocopa iris	4(4) Y	Hal	Lasioglossum villosulum	5(4) Y
And	Andrena hesperia	5(4) Y	Api	Xylocopa violacea	2(2) Y	Hal	Nomiapis diversipes	1(1) Y
And	Andrena lepida	3(1) Y	Col	Colletes brevigena	1(1) N	Hal	Seladonia gemmea	5(5) Y
And	Andrena miegiella	3(2) Y	Col	Colletes marginatus	5(4) Y	Hal	Seladonia smaragdula	5(4) Y
And	Andrena morio	5(4) Y	Col	Colletes nigricans	1(1) Y	Hal	Seladonia subaurata	5(4) Y
And	Andrena nigroaenea	5(3) Y	Col	Colletes similis	5(5) Y	Hal	Sphecodes alternatus	1(1) Y
And	Andrena nigroolivacea	5(4) Y	Col	Colletes succinctus	4(3) Y	Hal	Sphecodes gibbus	1(1) N
And	Andrena ovatula	2(2) Y	Col	Hylaeus annularis	2(1) Y	Hal	Sphecodes puncticeps	1(1) Y
And	Andrena pelluscens	1(1) N	Col	Hylaeus clypearis	5(5) Y	Hal	Vestitohalictus vestitus	2(1) Y
And	Andrena pilipes	4(4) Y	Col	Hylaeus deceptorius	5(5) Y	Meg	Anthidiellum strigatum	5(4) Y
And	Andrena sardoa	5(4) Y	Col	Hylaeus imparilis	5(5) Y	Meg	Anthidium taeniatum	1(1) N
And	Andrena similis	5(5) Y	Col	Hylaeus punctatus	4(3) Y	Meg	Coelioxys afra	1(1) Y
And	Andrena thoracica	2(0) Y	Col	Hylaeus taeniolatus	5(5) Y	Meg	Heriades crenulata	5(5) Y
And	Andrena variabilis	1(1) N	Col	Hylaeus variegatus	1(1) N	Meg	Heriades rubicola	5(4) Y
And	Andrena vetula	4(3) Y	Hal	Halictus brunnescens	2(1) N	Meg	Heriades truncorum	1(1) N
And	Andrena vulpecula	5(5) Y	Hal	Halictus fulvipes	4(3) Y	Meg	Hoplitis bisulca	5(4) Y
And	Andrena wilkella	2(2) Y	Hal	Halictus langobardicus	5(4) Y	Meg	Hoplitis fasciculata	2(2) N
And	Panurgus corsicus	5(5) Y	Hal	Halictus quadricinctus-brunnescens	3(3) Y	Meg	Megachile albisecta	1(1) Y
Api	Apis mellifera	5 (5) Y	Hal	Halictus scabiosae	5(4) Y	Meg	Megachile schmiedeknechti	5(5) Y
Api	Amegilla quadrifasciata	4(3) Y	Hal	Lasioglossum albocinctum	5(3) Y	Meg	Megachile centuncularis	5(4) Y

Api	Amegilla talaris	1(0) Y	Hal	Lasioglossum bimaculatum	5(4) Y	Meg	Megachile concinna	2(2) Y
Api	Anthophora crinipes	1(1) N	Hal	Lasioglossum brevicornis	1(1) N	Meg	Megachile leachella	1(1) N
Api	Anthophora dispar	5(5) Y	Hal	Lasioglossum griseolum	1(1) N	Meg	Megachile melanopyga	5(5) Y
Api	Anthophora nigrovittata	3(2) Y	Hal	Lasioglossum interruptum	5(4) Y	Meg	Megachile sicula	5(5) Y
Api	Anthophora sichelii	5(4) Y	Hal	Lasioglossum laterale	1(1) N	Meg	Osmia caerulescens	5(5) Y
Api	Bombus pereziellus	2(2) Y	Hal	Lasioglossum lativentre	3(3) Y	Meg	Osmia ferruginea	4(4) Y
Api	Bombus ruderatus	2(2) Y	Hal	Lasioglossum leucozonium	3(2) Y	Meg	Osmia latreillei	2(2) Y
Api	Bombus xanthopus	5(5) Y	Hal	Lasioglossum limbellum	1(1) N	Meg	Osmia ligurica	1(1) N
Api	Ceratina cucurbitina	1(1) Y	Hal	Lasioglossum lineare	1(1) N	Meg	Osmia nasoproducta	4(4) Y
Api	Ceratina cyanea	5(4) Y	Hal	Lasioglossum malachurum	5(3) Y	Meg	Osmia niveata	5(5) Y
Api	Ceratina dallatorreana	1(1) Y	Hal	Lasioglossum medinai	1(1) N	Meg	Osmia rufohirta	5(5) Y
Api	Ceratina parvula	3(0) Y	Hal	Lasioglossum morio	1(0) N	Meg	Osmia signata	1(0) Y
Api	Eucera nigrescens	5(2) Y	Hal	Lasioglossum nigripes	2(2) Y	Meg	Osmia submicans	2(2) Y
Api	Eucera nigrifascies	5(0) Y	Hal	Lasioglossum nitidulum	5(5) Y	Meg	Osmia versicolor	1(1) N
Api	Eucera nigrilabris	5(4) Y	Hal	Lasioglossum pauperatum	2(2) Y	Meg	Pseudoanthidium cf.leucostoma	1(1) N
Api	Eucera numida	4(0) Y	Hal	Lasioglossum pauxillum	2(2) Y	Meg	Rhodanthidium septemdentatum	4(4) Y
Api	Nomada goodeniana	1(1) N	Hal	Lasioglossum prasinum	5(4) Y			
Api	Nomada numida	3(2) N	Hal	Lasioglossum puncticolle	5(4) Y			

Table A2: type, size and sample size (N), average mass and standard error of measured pins

Pin mark and size	N	M (mg)	Std.err. M (mg)
Kabourek 0	9	21.5	0.2
Kabourek 1	29	37.2	0.3
Kabourek 2	10	46.6	0.2
Kabourek minuciae (5x)	10	0.67	0.02
Karlsbader 0	10	37.4	0.2
Karlsbader 1	10	38.0	0.2
Austerlitz 0	10	29.3	0.2
Austerlitz 1	10	36.8	0.4
Austerlitz 2	10	46.7	0.3
Sphinx 1	10	37.8	0.2

Table A3: landmarks and morphometric traits with procedure for the calculation

Tagma	Landmarks
Head – Lateral view	H_{L1}: posterior end of the compound eye; H_{L2}: anterior end of gena; H_{L3}, H_{L4}: dorsal end of compound eye and ventral end of the gena (maximum thickness).
Thorax – Lateral view	T_{L1}: anterior end of tegula; T_{L2}: ventral end of suture between thorax and 2nd coxa.
Abdomen – Lateral view	A_{L1}: anterior end of T1; A_{L2}, A_{L3}: dorsal posterior ends of T1, T2; A_{L4}, A_{L5}: ventral posterior ends of T1, T2 (intersection of T1/2 sutures with S1/S2 sutures).
Head – Dorsal view	H_{D1}, H_{D2}: points of minimum distance between posterior ends of compound eyes.
Thorax – Dorsal view	T_{D1}: anterior end of medial suture of scutum; T_{D2}, T_{D3}: proximal posterior ends of tegulae; T_{D4}: medial point of suture between scutellum and metanotum;
Abdomen – Dorsal view	A_{D1}: posterior visible end of ligament between thorax and abdomen; A_{D2}, A_{D3}: left and right distal ends of suture between T1 and T2; A_{D4}, A_{D5}: left and right distal ends of suture between T2 and T3; A_{D6}, A_{D7}: medial points of sutures between T1/T2 and T2/T3.

Tagma	Morphometric traits
Head	**H_{AP} = dist (H_{L1}, H_{L2})** **H_{LR} = dist (H_{D1}, H_{D2})** **H_{DV} = dist (H_{L3}, H_{L4})**
Thorax	**T_{AP} = dist (T_{D1}, T_{D4})** **T_{LR} = ITD = dist (T_{D2}, T_{D3})** **T_{DV} = dist (T_{L1}, T_{L2})**
Abdomen	C_0 = mean (dist(A_{D1}, A_{D2}), dist(A_{D1},A_{D3})) W_1 = dist (A_{D2}, A_{D3}) W_2 = dist (A_{D4}, A_{D5}) L_1 = sqrt (C_0^2 - $(W_1/2)^2$) L_2 = mean (dist(A_{D6}, A_{D7}), dist(A_{L4},A_{L5})) **$A_{AP} = L_1 + L_2$** **A_{LR} = mean (W_1, W_2)** C_1 = mean (dist(A_{D6}, A_{D2}), dist(A_{D6},A_{D3})) C_2 = mean (dist(A_{D7}, A_{D4}), dist(A_{D7},A_{D5})) S_1 = sqrt (C_1^2 - $(W_1/2)^2$)

$S_2 = \mathrm{sqrt}\ (C_2^2 - (W_2/2)^2)$

$\mathbf{A_{DV} = mean\ (S_1, S_2)}$

H = head; T = thorax; A = abdomen; AP = anteroposterior; DV = dorsoventral; LR = left-right; D = dorsal; L = lateral

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