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The Vitruvian spider: Segmenting and integrating over different body parts to describe ecophenotypic variation

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Abstract

Understanding what drives the existing phenotypic variability has been a major topic of interest for biologists for generations. However, the study of the phenotype may not be straightforward. Indeed, organisms may be interpreted as composite objects, comprising different ecophenotypic traits, which are neither necessarily independent from each other nor do they respond to the same evolutionary pressures. For this reason, a deep biological understanding of the focal organism is essential for any morphological analysis. The spider genus *Dysdera* provides a particularly well-suited system for setting up protocols for morphological analyses that encompass a suit of morphological structures in any nonmodel system. This genus has undergone a remarkable diversification in the Canary Islands, where different species perform different ecological roles, exhibiting different levels of trophic specialization or troglomorphic adaptations, which translate into a remarkable interspecific morphological variability. Here, we seek to develop a broad guide, of which morphological characters must be considered, to study the effect of different ecological pressures in spiders and propose a general workflow that will be useful whenever researchers set out to investigate variation in the body plans of different organisms, with data sets comprising a set of morphological traits. We use geometric morphometric methods to quantify variation in different body structures, all of them with diverse phenotypic modifications in their chelicera, prosoma, and legs. We explore the effect of analyzing different combined landmark (LM) configurations of these characters and the degree of morphological integration that they exhibit. Our results suggest that different LM configurations of each of these body parts exhibit a higher degree of integration compared to LM configurations from different structures and that the analysis of each of these body parts captures different aspects of morphological variation, potentially related to different ecological factors.

KEYWORDS
Araneae, Canary Islands, Dysderidae, geometric morphometrics, integration

1 | INTRODUCTION

The study of how the phenotype can be altered by selection goes back to the experiments performed by Darwin before the publication of his book "On the origin of species" in 1859 (Andersson, 2009). However, adaptation is an inherently multivariate process and natural selection often acts upon sets of functionally related traits, rather than on unidimensional phenotypes (Blows, 2007; Lande & Arnold, 1983; Phillips & Arnold, 1989; Schluter & Nychka, 1994). Furthermore, not all evolutionary pressures necessarily drive the different characteristics of organisms toward the same direction. Under the definition of the species' fundamental niche by Hutchinson (1957) as an hypervolume of *n* dimensions, the existence of a species in a given environment is understood as being determined by its capacity to adapt to different ecological conditions, and its tolerance to these environmental settings is in turn influenced by its phenotypic attributes (Carscadden et al., 2017; Givnish, 1987). The suit of morphological features that improve an organism's performance in its environment is known as functional traits (McGill et al., 2006; Nock et al., 2016). In this context, the multidimensional hutchinsonian niche of *n* ecological dimensions is also expected to translate into a multidimensional space of different functional morphological traits (Eklöf et al., 2013) that define the phenotypic properties of the species, such as its external shape. Therefore, to understand the morphological differentiation among different species, a multidimensional phenotypic approach is needed (Guillerme, 2018), and to infer the evolutionary pressures that have driven such differentiation, we need to know how phenotypes and their function relate to their ecological environment. The mutual links between these organismal properties and their evolution are summarized in the ecomorphological paradigm (Arnold, 1983). However, understanding the relationship between phenotypic characters, ecological variables, and the effect of phenotypic variation on fitness is not always straightforward. While predictions can be made based on our knowledge of how organisms work, linking patterns of variation in different traits with different ecological pressures in non-model systems may not be clear. Furthermore, depending on the question to be addressed, one may be interested in one particular structure, a set of them, or the entire body, and thus the challenge emerges on how to best choose which traits to investigate and how to combine the information derived from each of them (Guillerme et al., 2020).

Additionally, those ecological-related phenotypes shaped under different evolutionary pressures are not completely independent from each other, because organisms are integrated to function as a whole (Klingenberg, 2009). However, the level of covariation between them could vary depending on the relationships between the different body parts (Olson & Miller, 1958). Traits with higher levels of covariation (i.e., integration), than others, form composite units (modules) affected by more similar evolutionary pressures compared to other body parts. These integrated subunits are interconnected conforming, the whole body of an organism that needs to work in a coordinated manner, where changes in one trait could be inevitably accompanied by changes in others (Adams, 2016).

This integration of different modules has different reasons: genetic, developmental, functional, or evolutionary (Klingenberg, 2008). One of the consequences of trait integration is that determining whether certain phenotypes have been shaped by certain evolutionary pressures or whether, on the contrary, trait variation emerges as a consequence of the coevolution with other interconnected modules is not always straightforward. In this sense, understanding which sets of traits show high covariation is an important aspect to discern the different biological factors that may explain their evolution.

Organisms with a modular body plan, where different structures can be intuitively associated with particular ecological or social functions, and thus predicted to respond to specific evolutionary pressures, provide excellent study cases for establishing how morphological variation can be described quantitatively. In addition, they ease the association of such functional structures to its underlying causes, when previous knowledge is scarce. Spiders, and arachnids in general, are one such case. From the comparative morphology of the chelicera musculature (Wood & Parkinson, 2019) to the use of body size to understand ecophysiology (Canals et al., 2015), most previous studies on spiders have been directed at understanding the function and meaning of a specific character related to a specific adaptation in different sets of species. More recently, however, an increasing number of studies has focused on establishing a standardized framework to investigate different functional traits in spiders (Lowe et al., 2020; Macías-Hernández et al., 2020), highlighting the raised attention to the relation with the ecological and morphological characters in this group of organisms. However, despite the relatively obvious functional implications of some phenotypic characters (e.g., legs for locomotion or social interactions, chelicerae for feeding, etc.), a detailed protocol for obtaining high-resolution morphological data on different body structures and an integrated assessment of such variation with relation to different factors is still not available for spiders.

The inherent complexity of certain body structures is better approached for morphological studies if shape information is considered (Klingenberg, 2010). In technical terms, one major tool for studying the variation in the shape of body parts and the covariation between them (i.e., integration) is landmark (LM)-based geometric morphometrics (GMs). These methods differ from traditional morphometric approaches in how shape information is obtained, as they capture the geometry of the morphological structure of interest, and preserve it throughout the analyses (Adams et al., 2004). These tools have expanded the way we visualize and study shape variation, providing a fine-scale description of morphological structures. However, despite the statistical strength of GM in characterizing the shape of morphological structures, few studies have applied this morphometric technique in spiders. Previously, LM-based analyses have been limited to characterizing genitalic variation across species (Crews, 2009) and intraspecific allometry in sexual dimorphism (Fernández-Montraveta & Marugán-Lobón, 2017; Kallal et al., 2019), and delimiting and identifying species overlooked with traditional methods (Wilson et al., 2021). Thus, although morphological studies on spiders have been extensive, we lack fine-tuned

protocols for shape data acquisition and a detailed knowledge of the variation of different body parts and how these are integrated with each other.

The spider genus *Dysdera* Latreille, 1804, also known as woodlouse-hunter or red devil spiders, has a western Palearctic distribution, with Macaronesia as its westernmost limit (Arnedo & Ribera, 1999), and to date around 300 species have been described (World Spider Catalog, 2022). Of nocturnal habits, these species are usually found under rocks, barks, or dead logs (Macías-Hernández et al., 2008) (Figure 1a). The genus exhibits a high interspecific variability in their cheliceral mouthparts, which has been related with different levels of trophic specialization in feeding on isopods, that is,

oniscophagy (Řezáč & Pekár, 2007), where some more specialized species exhibit varying degrees of preference toward capturing isopods over other arthropod preys, while other species retain a rather generalist diet (Toft & Macías-Hernández, 2021). For this reason, *Dysdera* species have been object of several studies that tried to associate variation in cheliceral shape with their ecological performance (e.g., Řezáč & Pekár, 2007; Řezáč et al., 2021; Toft & Macías-Hernández, 2017, 2021). This genus has diversified extensively in the Canary Islands with numerous endemic species across the seven major islands. In addition to the different levels of oniscophagy, other remarkable adaptations have been recorded. Indeed, the Canary Islands harbor several obligate cave-dwelling
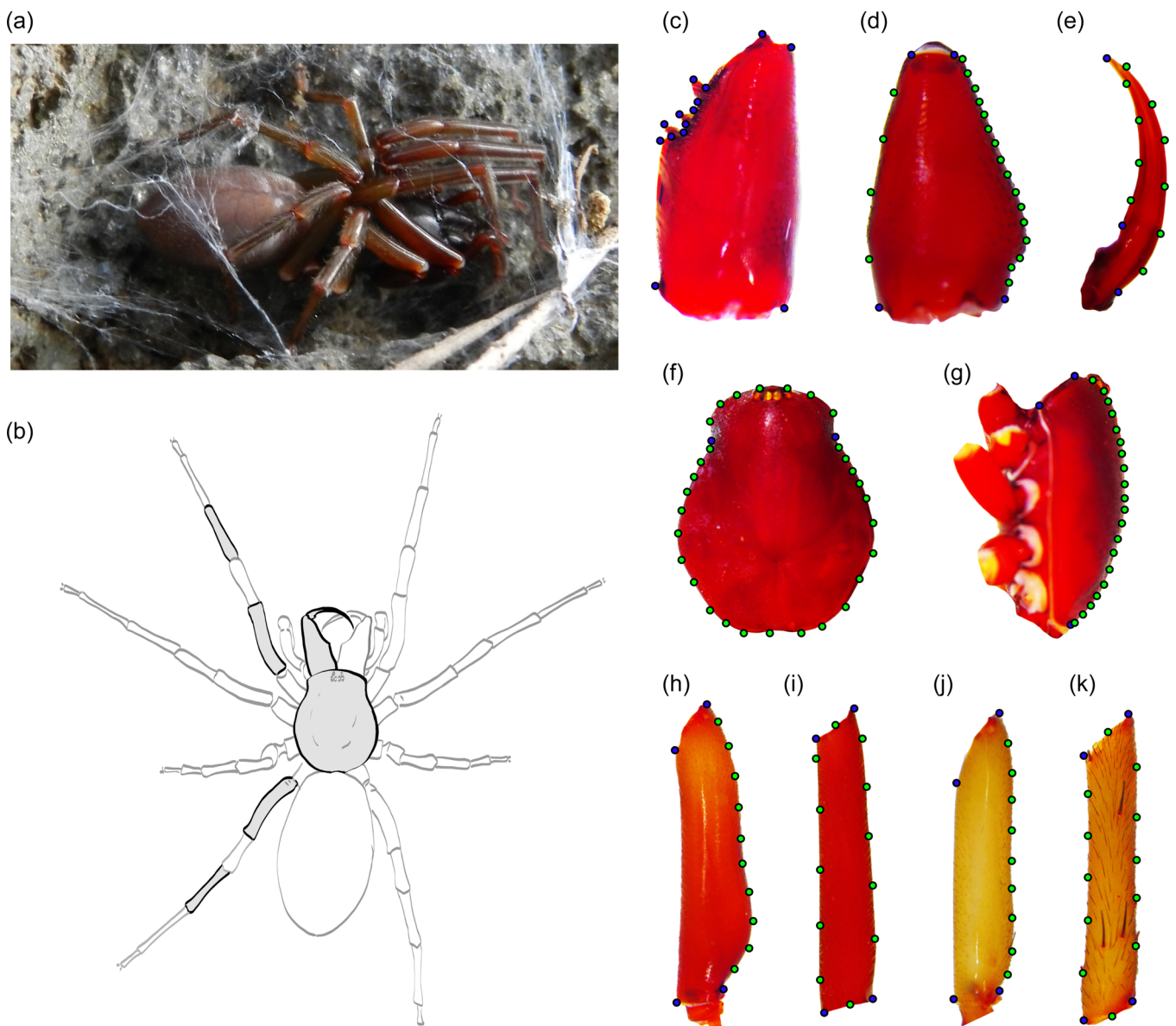
FIGURE 1 Analyzed landmark configurations of the *Dysdera* spider species and digitized landmarks. Blue dots represent fixed landmarks and green dots represent semilandmarks. (a) *Dysdera silvatica* in a typical silk retreat under the rock; (b) different body parts analyzed in the *Dysdera* species of this study (highlighted in gray); (c) ventral view of the chelicera (Q1); (d) lateral view of the chelicera (Q2); (e) ventral view of the fang (Q3); (f) dorsal view of the prosoma (C1); (g) lateral view of the prosoma (C2); (h) lateral view of the leg 1 femur (L11); (i) lateral view of the leg 1 tibia (L14); (j) lateral view of the leg 4 femur (L41); (k) lateral view of the leg 4 tibia (L44).

species that exhibit remarkable somatic modifications (Arnedo et al., 2007), while some epigean spiders exhibit modifications in the shape of the carapace (Arnedo & Ribera, 1999). Due to these ecological particularities (trophic specialization and cave adaptation), the *Dysdera* species from the Canary Islands constitute a well-suited model for exemplifying high-resolution protocols for quantifying variation in different phenotypic traits and their integration in spiders and other arachnids.

The aims of the present study are to: (1) provide a practical guide for describing shape variation in spiders using GM to integrate information from different body parts; (2) to provide a first assessment on which of these structures may be more informative for exploring phenotypic variation related to particular ecological pressures; (3) to investigate whether morphological variables derived from the same or different body structures tend to covary strongly, or if, by contrast, they may respond independently to different selective pressures; and (4) to provide a practical guide on how information on different body structures may be combined to provide a global or partial view of morphological variation. To this end, we first establish a standardized protocol for obtaining data on the shape variation of different sides and perspectives of the chelicera, legs, and prosoma using two-dimensional LM-based GMs. Due to their function, and if integration mostly occurs across LM configurations of each structure, with different body structures being relatively free to vary independently, we expect that for some body parts (e.g., chelicera, legs) a clear differentiation will occur among species that are also markedly divergent in the dimensions of their niche relevant for that body part (e.g., trophic ecology and cave-dwelling, respectively; see also below). In this case, the distribution of species in global morphospace (i.e., considering all different LM configurations of all studied body structures) may exhibit high levels of "noise," due to the combination of different ecological influences driving different parts of the phenotype in different directions. If, by contrast, different body structures are highly integrated, variation across individuals and species in the global morphospace would rather be dominated by that ecological factor most strongly influencing the phenotype, and it would align quite closely to the partial morphospace that corresponds to the body structure with a direct functional link to such dominant ecological factor. Similarly, to the extent that certain species are morphologically differentiated across several of these ecological axes of interest, a combination of different body structures may better capture this joint differentiation. By contrast, species that are only differentiated in specific axes may become more blurred when considering several body structures together.

2 | MATERIALS AND METHODS

2.1 | Specimens used

All specimens examined were collected during field campaigns conducted by the authors and colleagues during the last 30 years

and are available at the Centre de Recursos de Biodiversitat Animal of the Universitat de Barcelona (CRBA) and the collection of the Departamento de Zoología de la Universidad de La Laguna, Tenerife, Canary Islands (DZUL). Individuals were captured by active searching under rocks, logs, and tree barks. The captured specimens were either preserved in 76% or 95% EtOH and, in some cases, stored at −20°C for subsequent molecular analyses at the Universitat de Barcelona. All specimens were collected following institutional and governmental regulations and the permits for all species captured were granted by the local authorities of each island or by the governing body of each natural reserve (Cabildos of El Hierro, La Gomera, La Palma and Tenerife, Garajonay and Caldera del Taburiente National Parks).

2.2 | Model system

We selected a suit of *Dysdera* species from the Canary Islands that exemplify different ecological habits or that exhibit markedly different phenotypic structures. Note that our objective when selecting these species was not to provide a full assessment of morphological variation in Canarian *Dysdera* and its relation to ecology. Such an endeavor would require a much more comprehensive sampling of species and would also need to take phylogenetic relationships into account and perform formal statistical assessments using phylogenetic comparative methods to assess the relative contribution of adaptation and shared evolutionary history in shaping the evolution of ecomorphological functional traits. However, such an objective lies beyond the scope of this study. Instead, here we aimed at including representatives with obviously different ecological and morphological properties, such as to optimize the technical and analytical protocols for exploring shape variation of different body parts in spiders. For this reason, we selected species representative of extreme morphologies and ecologies, with no consideration whatsoever of their phylogenetic position and relationships, while also including sampling of intraspecific variation (for *D. silvatica*, see below).

We considered three species that occur exclusively in cave environments: *Dysdera unguimmanis* Ribera, Fernandez and Blasco (1986), which exhibits extreme phenotypic cave adaptations (eye loss, depigmentation, and appendage elongation; Figure 2c); *D. ambulotenta* Ribera, Fernandez and Blasco (1986), eyeless and showing appendage elongation, but preserving dark reddish pigmentation in the carapace; and *D. ratonensis* Wunderlich (1992), which shows markedly reduced eye size but no other obvious somatic adaptation to the underground environments. Additionally, we considered species that represent ecomorphs with different trophic adaptations (Toft & Macías-Hernández, 2021). Specifically, we included two species related to a generalist diet with unmodified chelicera, *D. verneui* Simon (1883) (Figure 2a) and *D. silvatica* Schmidt (1981) (Toft & Macías-Hernández, 2021). Recent studies (Řezáč et al., 2021) have defined these two species as oniscophagous, a feeding preference, which could be more related to specialization.
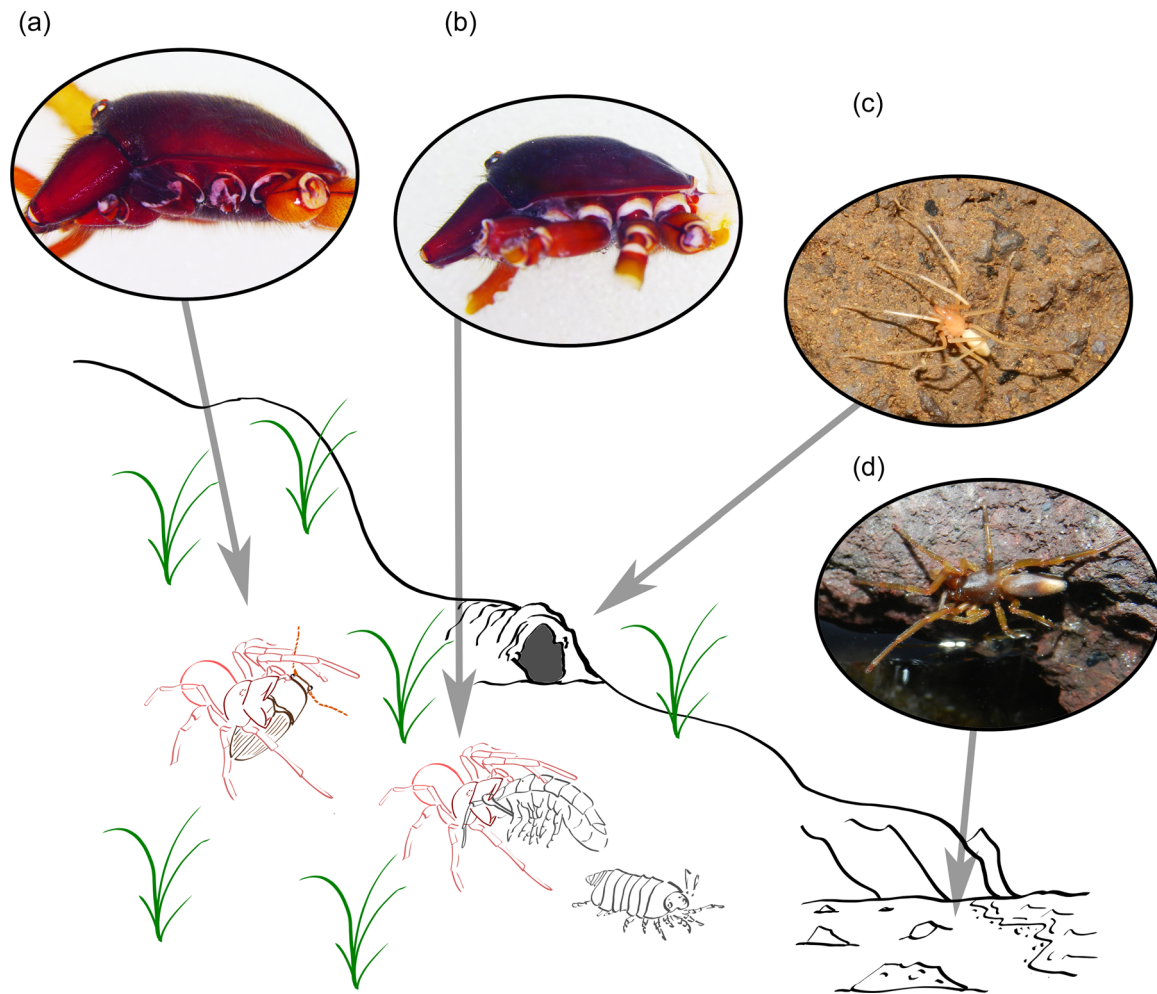
FIGURE 2 Different ecological groups selected for the study. (a) Generalist species; (b) *Onsicophagous* species; (c) cave-dwelling species with troglomorphic adaptations; (d) species adapted to intertidal environments.

However, note that the experiments conducted by Řezáč et al. (2021) did not consider the level of trophic adaptation, but rather focused on the tactic used to prey on isopods or on whether these species avoid predating this arthropod. For this reason, we considered it more appropriate to treat them as species with a generalist diet. Additionally, to also include some population-level phenotypic variation in our data set, *D. silvatica* was represented by specimens coming from populations in three different islands (La Gomera, El Hierro, and La Palma). Specimens of each island were analyzed separately. We also included in our data set species that present deviant cheliceral morphologies: *D. insulana* Simon (1883), which carries chelicera with concave-shaped paturon (the basal segment, in lateral view; Figure 2b) and *D. ramblae* Arnedo, Oromí and Ribera (1997), which shows a short and dorsoventrally flattened chelicera fang, both specialized on isopod consumption with different hunting tactics (Řezáč et al., 2008). Similarly, *D. breviseta* Wunderlich (1992) and *D. macra* Simon (1883) carry chelicerae with slightly elongated paturon and have also been associated to a specialist diet, but in addition, these species present a marked bulge in the carapace (in lateral view, referred as step-shaped carapace;

Toft & Macías-Hernández, 2017). Finally, *D. curviseta* Wunderlich (1987) was chosen because it is ecologically quite distinct to the previous species, as it occurs in intertidal environments (Arnedo & Ribera, 1999; Macías-Hernández et al., 2010; Figure 2d). Previous studies have failed to show differences in the somatic morphology between males and females in some *Dysdera* species (Cooke, 1965); for the purpose of the present work, all specimens of the same species had been analyzed indistinctively regarding their gender.

2.3 | GM data acquisition

We took high-resolution photographs of different body parts of all specimens with a digital camera LEICA DFC 450 attached to a LEICA MZ16A stereoscopic microscope using Leica Application Software v.4.4 (Leica Microsystems Ltd.). We photographed nine different perspectives (Figure 1b): three of the chelicera (ventral and lateral side of the paturon, and the ventral side of the fang, Q1, Q2, Q3, respectively; Figure 1c–e), two of the prosoma (dorsal and lateral, C1 and C2; Figure 1f,g) and four perspectives of two different

segments of the first and fourth let (the retrolateral femur and the retrolateral tibia, L11, L41, L14, and L44, respectively; Figure 1h–k). Whenever possible we used the left chelicera and leg of five females and five males of each species. When this was not possible due to broken parts or bad preservation, we assumed symmetry in the vertical plane and the right side was digitized and reflected. A total of 91 specimens comprising 10 different *Dysdera* species from the Canary Islands were photographed for this study. All photographs of each structure perspective were assembled using the software TpsUtil (Rohlf, 2015) and landmarks and semilandmarks were recorded (Figure 1c–k) using TpsDig2 (Rohlf, 2015). Each landmark configuration was then subjected to a Generalized Procrustes Analysis (Gower, 1975; Rohlf & Slice, 1990) using the function *gpagen* to remove nonshape variation related to scaling, position, and rotation from landmark coordinates and obtain shape variables. During superimposition, the position of semilandmarks was optimized by minimizing bending energy. We used the statistical environment R (R Core Team, 2021) and the R package "geomorph" (Adams et al., 2021; Baken et al., 2021) to conduct all data analyses.

2.4 | Integration of different landmark configurations and structures

To examine the covariation of the different LM configurations of each anatomical structure, we grouped them into three subsets according to the morphological structure they represent (i.e., three LM configurations of the chelicera, the dorsal and lateral LM configurations of the carapace, and four LM configurations of distinct leg segments). A pairwise integration test was performed between all pairs of LM configurations belonging to the same subset and across all pairs of LM configurations of different anatomical aggrupation. We used the function *integration.test* to test for morphological integration between the different LM configurations by performing a partial least squares (PLS) analysis. Then, to test whether morphological integration was stronger between LM configurations of the same structure, as compared to across structures, we extracted effect sizes (*z*-scores) of the aforementioned pairwise PLS analyses as an estimate of the strength of integration of each pair (Adams & Collyer, 2016). Then, we used an analysis of variance (ANOVA) comparison based on 999 random permutations as applied through the function *lm.rrpp* of the RRPP R-package (Collyer & Adams, 2018, 2021) to evaluate whether within-structure pairs of LM configurations exhibited higher levels of integration compared to between-structure pairs.

2.5 | Patterns of phenotypic variation across structures

With the function *combine.subsets*, we gathered all different LM configurations belonging to the same morphological structure in combined data sets. We also created a subset combination with all different LM configurations of all structures together to explore the potential of analyzing all phenotypic information combined. For each subset, we performed a second generalized Procrustes analysis to scale all LM configurations to their unit-centroid size to correct their proportions (Collyer et al., 2020; Stepanova & Womack, 2020). With each of the resulting subset combinations, we conducted two different principal component analyses (PCAs) using the function *gm.prcomp*. The first analysis was performed using the mean coordinates of each species to obtain a clearer view of the relative position of the species' average shape in morphospace. The second analysis used all (previous) analyzed specimens of each species to allow us to inspect intraspecific disparity with each subset combination. We then plotted the first two principal components of the resulting PCAs for each subset to visualize differences in species and individual morphotype occupancy and disparity across LM configuration/structure combinations. To quantify the percentage of total shape variation represented by each separate subset, we first calculated pairwise Euclidean distances between observations (i.e., species means or individual shapes) in each of the considered morphospaces (i.e., global, chelicerae, carapace, limbs). We then performed a Mantel test of matrix association between each of the structures to the subset including all structures. Shape variation across PC axes was visualized through deformation grids produced using the function *plotRefToTarget*. To investigate whether major directions of shape variation as captured by PC axes were concordant at the species and individual levels, we calculated the angle observed between the species-PCA and the individual-PCA vectors and then used a permutation procedure to test whether this angle was significantly different than zero, which would imply a perfect alignment of vector directions (Martínez-Gil et al., 2022).

3 | RESULTS

3.1 | Integration of phenotypic landmark configurations and structures

Integration tests between combinations of different LM configurations and structures indicated significant integration across all pairs (all *p* < .01; Supporting Information: Table S1), which varied extensively in strength depending on the considered pair (Figure 3a). The ANOVA comparison suggested that pairs that encompassed different LM configurations of the same morphological structure exhibited overall higher levels of integration than those that included LM configurations belonging to different structures (*z* = 2.4168, *p* = .003; Figure 3b).

3.2 | Morphospace organization across data subsets

When examining the morphospaces of the different subset combinations derived by the PCA conducted at the species level,
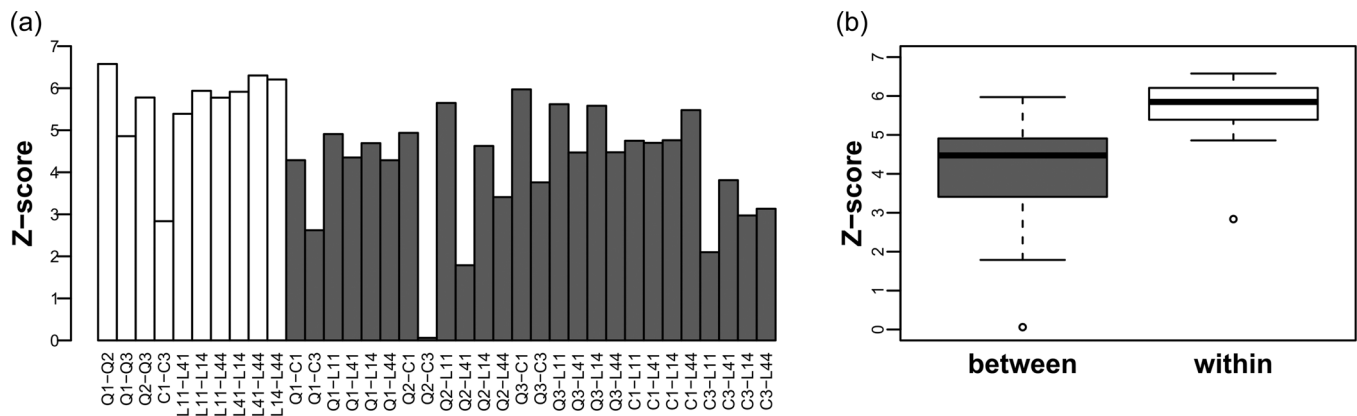
FIGURE 3 Pairwise integration values (a) and overall levels of integration (b) of landmark (LM) configurations related to the same morphological structure (white) versus LM configurations of different morphological structures (gray).

some common patterns could be observed. For instance, the sampled populations of the generalist species *D. silvatica* from different islands and the also generalist *D. verneaui* were always close together. Similarly, the cave-adapted species *D. ratonensis* and the intertidal *D. curviseta* also occupy nearby locations in all examined morphospaces. When all phenotypic LM configurations were combined (Figure 4a), PC1 showed the greatest explained variability (78.2%) of all the different subsets. This is most likely due to the marked differentiation of *D. unguimanis*, the species with the most marked cave adaptations, since the remaining species all clustered together in this morphospace. Considering the subset combination of the different LM configurations of the chelicera (Figure 4b), PC1 explained 55.6% of the total variance, while PC2 explained 16.1%. The two extremes of PC1 were occupied by *D. insulana* and *D. ramblae*, both of which exhibit distinct chelicera adaptations in the archipelago, that is, concave chelicera and flat fang, respectively. With the subset combination of the two different carapace LM configurations (Figure 4c), PC1 explained 40.3% and PC2 25.6% of the variance, which translated into a scattered distribution of the analyzed species. Species with a generalist diet (*D. silvatica* and *D. verneaui*) occupied the center of the morphospace, while the cavernicolous species were rather scattered across PC1. The two species with the step-shaped carapace (*D. breviseta* and *D. macra*) were differentiated from the rest across PC2. Interestingly, differentiation of other species not related to this carapace shape modification was also observed, as is the case of *D. ramblae*, a species with one of the most extreme modifications in the chelicera (flat fang). The proportion of variance explained by the two first principal components for the subset of all leg LM configurations (Figure 4d) was 55.2% and 25.6% (PC1 and PC2, respectively). Here, all surface species were grouped together; meanwhile, the cave dwellers (*D. ambulotenta*, *D. unguimanis*, and *D. ratonensisi*) and the intertidal species (*D. curviseta*) were separated from the rest. Interestingly, the intertidal *D. curviseta* and the cave-dwelling *D. ratonensisi* clustered close together, while the other cave dwellers, *D. ambulotenta* and

D. unguimanis, occupied opposite positions in the PC2 of this morphospace. The Mantel test revealed that the distance across species means of the combination of the different LM configurations of the legs were more strongly associated with that observed considering the global morphospace ($r = .68$, $p = .012$ for leg LM configurations combination; $r = .50$, $p = .004$ for the chelicera combination; $r = .48$, $p = .028$ for the carapace combination).

Morphospaces derived from individual-level PCAs (Figure 5), provided additional information on the intraspecific morphological variation as captured by different body structure combinations. Species' morphological properties appeared better defined when using all character subsets together, where individuals of each species were much more tightly packed, occupying a reduced area in the morphospace (Figure 5a). By contrast, when considering each character subset separately, a wider overlap between species was observed. In this case, each of the separate character subsets captured a visibly lower amount of the variation represented by the global combination of all subsets compared to the species-level analysis, although the leg LM configuration subset was again the one most highly correlated to the combination of all structures (Mantel test: for the chelicera, $r = .26$, $p = .001$; for the carapace, $r = .39$, $p = .001$; for the legs, $r = .45$, $p = .001$).

3.3 | Shape variation captured

Shape variation described by PC1 axes aligned between individual and species-level variation only for the limbs ($\theta = 8.85$, $p = 1$), but it expressed slightly different aspects when considering both hierarchical levels for the remaining LM configurations ($\theta = 10.13$, $p = .001$ for all different LM configurations combined; $\theta = 15.86$, $p = .001$ for cheliceral LM configurations; $\theta = 53.22$ $p = .029$ for carapace LM configurations). Focusing on species-level variation, however, deformation grids describing the shape variation observed across the first two principal components when considering all the different LM configurations combined (Figure 6)
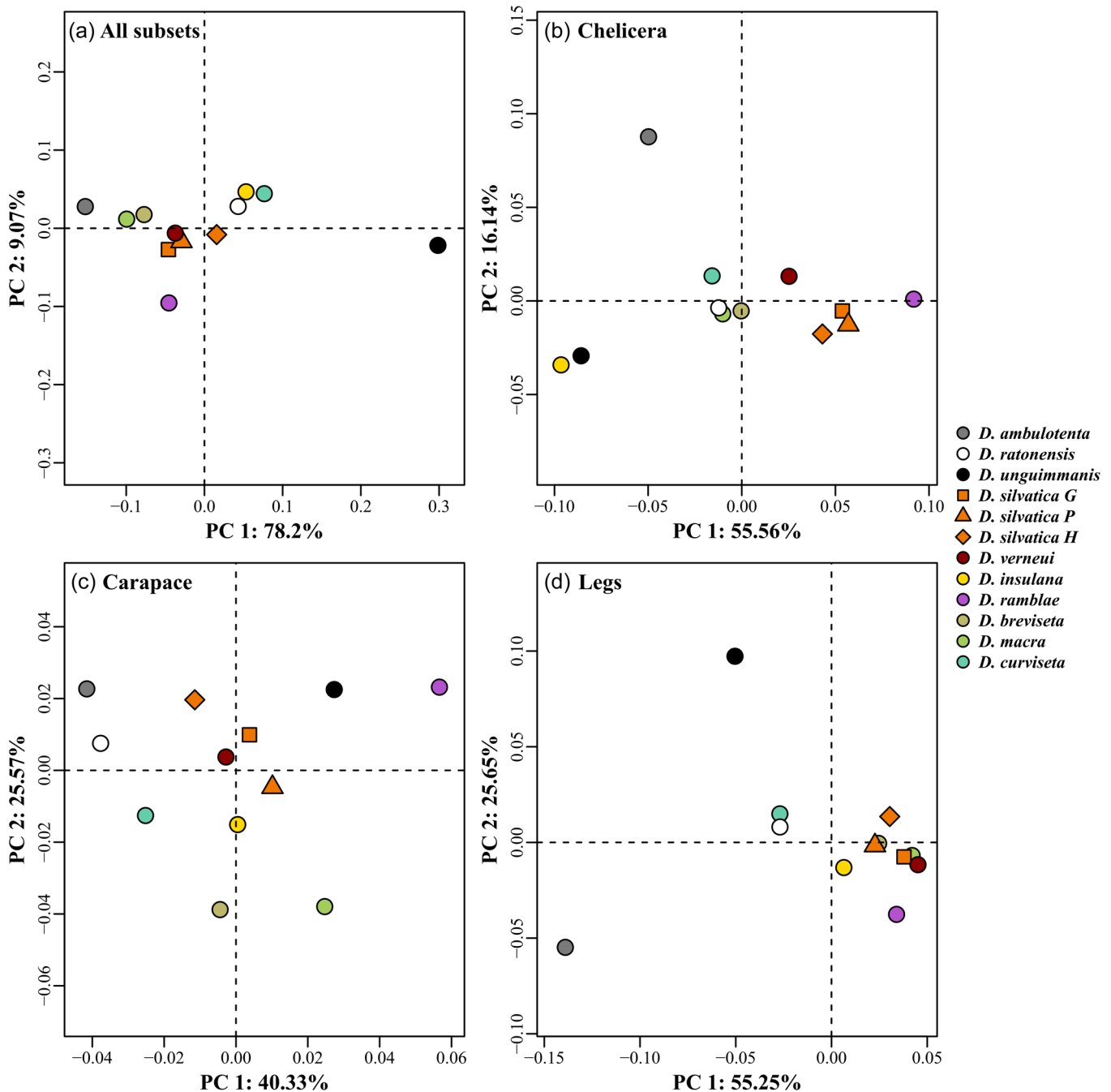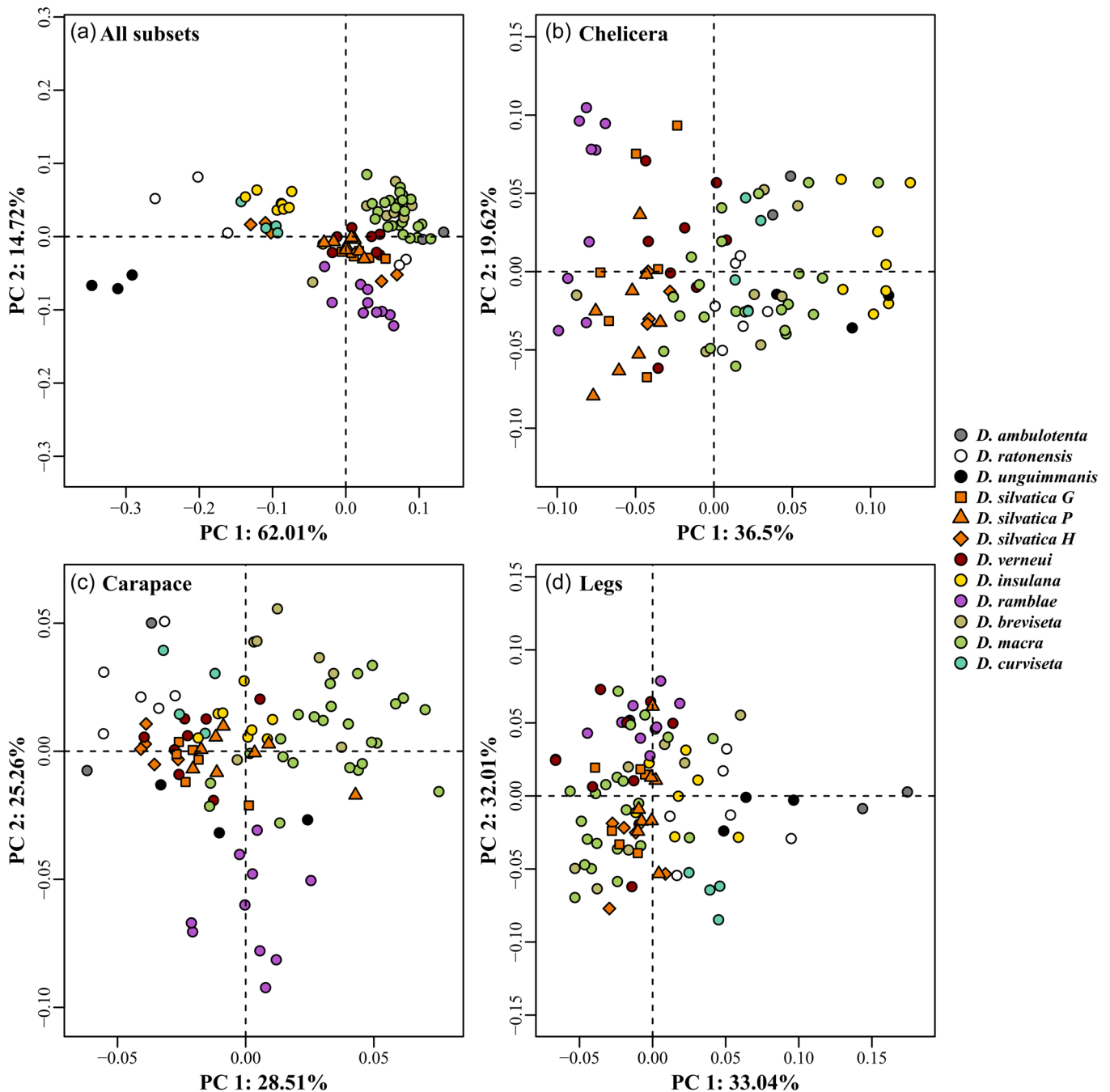
FIGURE 4 Phenotypic space of species means for the different subset combinations. (a) Subset of all landmark (LM) configurations combined. (b) Subset of all chelicera LM configurations (Q1, Q2, and Q3). (c) Subset of all prosoma LM configurations (C1 and C2). (d) Subset of all leg LM configurations (L11, L14, L41, and L44).

seemed to express fairly similar patterns of shape variation as those obtained by analyzing each structure separately, albeit with less intensity.

Focusing on the subset of the chelicera, major directions of shape variation were noticeable in the apical part of the ventral view, which got enlarged or retracted distally, increasing or decreasing the distance between the cheliceral teeth and the distal part of the groove, and in the central part of the fang, making this appendix shorter and wider or more elongated and thinner. Both modifications

were largely driven by the two species on the extremes of the PC1 of these LM configurations, *D. insulana* and *D. ramblae*, both specialist species with distinct cheliceral modifications—elongation of the chelicera and the fang, and a stouter chelicera and flattened fang, respectively. With the subset of the carapace, shape variation was circumscribed to the frontal part in the dorsal LM configuration, making it wider or narrower, and in the central part in the lateral LM configuration. However, shape variation across PC2 was markedly associated with the area of the carapace bulge characteristic of the

FIGURE 5 Phenotypic space of all specimens analyzed for the different subset combinations. (a) Subset of all landmark (LM) configurations combined. (b) Subset of all chelicera LM configurations (Q1, Q2, and Q3). (c) Subset of all prosoma LM configurations (C1 and C2). (d) Subset of all leg LM configurations (L11, L14, L41, and L44).

two step-shaped carapace species in the Canary Islands (*D. breviseta* and *D. macra*; Figure 6b). Finally, with the subset combination of the different leg LM configurations, the shape variation was visible across all the tibia, with an elongation or shortening of these different LM configurations, and in the basal and apical part of the femur. This elongation in the tibia was associated with the different species that are obligate cave dwellers, *D. unguimanis* and *D. ambulotenta*. The modification in the femur also conferred to this LM configuration a more slender or stouter shape.

4 | DISCUSSION

Although the study of phenotypes and how morphological variation is related to ecological performance is a field with a long history (e.g., Arnold, 1983), deciding which character is related to which ecological pressure and which traits are then worth investigating is not straightforward in nonmodel organisms. In the present study, we have shown that different LM configurations of the same or closely related structures exhibit

(a)

(b)
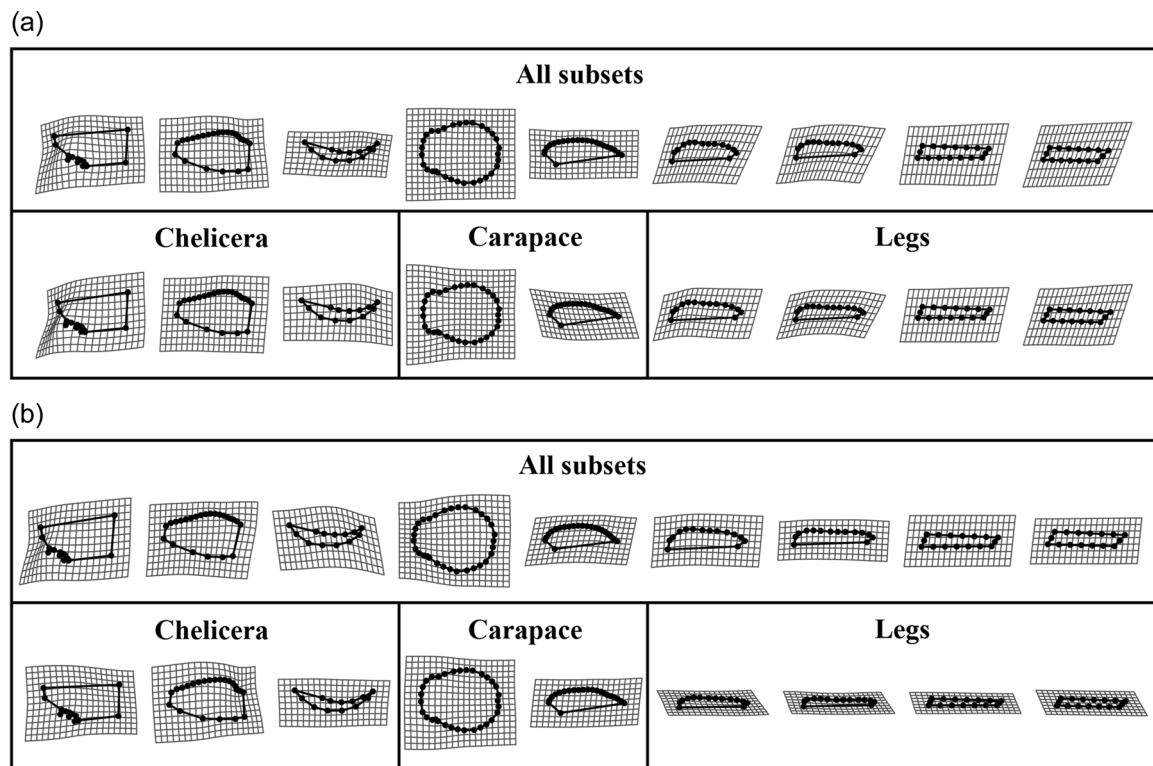
FIGURE 6 Deformation grids depicting shape differences between the minimum and maximum extremes of principal component (PC) axes for all the different LM configurations combined (top), and the subsets of the chelicera, carapace and legs (bottom) in PC1 (a) and PC2 (b).

higher levels of integration, allowing the study of 3D morphologies from the combination of 2D morphometric data acquisition, ensuring in this way the adequate analysis of these traits when technology or time availability are limiting factors. In general, examining each character set separately is more intuitive for linking morphological variation across species to potential underlying ecological pressures: this is the case, for instance, with the putative trophic meaning of the cheliceral morphospace; or with the phenotypic modifications of species adapted to subterranean environments, which are clearly differentiated across the first component of the leg morphospace. However, when all these characters are analyzed together, a greater cohesion of individual variation around species' means is achieved, providing higher resolution to describe the morphospace occupied by each species, without providing relevant information or species aggrupations related to any apparent ecological performance. This is concordant with previous studies focusing on characterizing ecological guilds in spiders, based on multivariate analysis of linear morphological traits, where a high overlap of the occupied trait space between different guilds was observed (Wolff et al., 2022). Based on this exploration, we provide a first assessment of the organization of shape variation in the chelicera, legs and prosoma of spiders, and some guidelines on how to choose which structures to analyze based on the biological question at hand.

4.1 | Phenotypic integration of landmark configurations and characters

For the proper function of complex phenotypes, traits must work together in a coordinated manner (Murren, 2012). From a methodological perspective, delimiting which traits can covary, and therefore function as morphological modules, in nonmodel organisms cannot be taken for granted. Here, we explore covariation patterns across different LM configurations representing Canarian *Dysdera* spider's body parts (i.e., the chelicera, carapace and limbs) to provide a simplified protocol that facilitates the choice of trait sets for future studies. Our analyses indicate that, in the empirical system explored here, the different phenotypic LM configurations analyzed exhibit significantly higher values of integration when they represent the same morphological structure. This confirms that the different data, acquired from a single body part, function well as a two-dimensional approximation of three-dimensional structures, thus providing a means of reducing the time and resources required for capturing and analyzing their shape (e.g., data acquired with computed tomography scans). Although some degree of integration is observed in all structures, as expected, our results highlight that different body parts work as more highly integrated modules that may vary independently to a certain extent, thus resolving the constraints imposed by a complete integration of different structures (Goswami et al., 2014). This is mirrored in the different patterns of distribution of species

across the morphospaces corresponding to each structure, where species with different ecological preferences seem to group depending on the character analyzed. In fact, the potential ecological significance of shape variation, which emerges across subset combinations (see below), together with the higher integration values among LM configurations related to the same body part (Figure 3), suggest that integration is constraining the variation of structures in certain directions (Goswami et al., 2014), most likely coordinating the evolution of functional units (Klingenberg, 2010).

4.2 | Trait integration, distribution of species across different morphospaces, and the meaning of shape variation

Differentiation within and across species in the morphospaces, obtained through PCA of different structural subsets (i.e., chelicera, legs, abdomen, total), provides preliminary hints on the potential ecological factors related to patterns of morphological variation. Despite the merely exploratory nature of this study, we have purposefully included species that exemplify extreme ecologies and morphologies to investigate how different morphological subsets may capture the relevant shape variation. Our results match intuitive expectations, based on the functions of different body parts, and add to our knowledge of how body shape variation in spiders may be best described using GMs.

Species distribution across the chelicera morphospace, comprising all different LM configurations of this structure, appears to reflect feeding habits. Species with different levels of oniscophagy are aggregated through the morphospace that represents variation in the chelicera shape (Toft & Macías-Hernández, 2021). This is not surprising as these species were precisely selected because of the high variability in their chelicera morphology that was previously suggested to be related to isopod predation (Řezáč & Pekár, 2007; Řezáč et al., 2021). Similarly, surface species are all phenotypically similar across the leg morphospace (Figures 4c and 5c), while cave-dwelling species are quite distinct phenotypically from all other *Dysdera*, a fact that drives variation across the leg-PC1. The distinctiveness of cave species in this morphospace also fits empirical observations, as one of the main adaptations to subterranean environments is the elongation of the legs (Christiansen, 1992; Deeleman-Reinhold & Deeleman, 1980). However, it is also interesting to note that the cave-dwelling species differ morphologically across PC2 (e.g., *D. ambulotenta* and *D. unguimanis*). These differences may hint at different levels or types of troglomorphic modifications, which could be either due to differences in the time of cave colonization or to the existence of distinct microhabitats within the subterranean environment (Arnedo et al., 2007; Mammola et al., 2020). Interestingly, some intermediate forms between species with marked troglomorphic adaptations and epigean species can also be observed (e.g., *D. ratonensis* and *D. curviseta*). In this regard, it is worth noting that although one of these species (*D. ratonensis*) has been exclusively collected in caves, it exhibits little evidence of

troglomorphism, except for a reduction of eye size, which varies according to the locality—being slighter in the north and more pronounced in southern caves, suggesting this species has only recently colonized the subterranean environment (Arnedo & Ribera, 1996). A more detailed study specifically quantifying the ecological habits of cave-dwelling species and the extent to which they may have affected their morphological properties and taking phylogenetic relationships into account to test for the differentiation between troglomorphic and epigean species would definitely add to our knowledge of how the structural habitat used by spiders may have shaped adaptive evolution of the legs in these organisms.

The stronger association observed between the leg shape and the global morphospace, both when examining species means and individual-level variation, could be explained by the marked distinctiveness of the highly adapted cave-dweller species, not only with the surface ones but also between them. Additionally, the leg subset combination is the only one for which we did not find significant differences in the direction of PC1 between individual and species-level patterns of shape variation. This is most likely due to the relative simplicity of these different LM configurations compared to the higher complexity in the chelicera or the carapace: in more complex structures across different data sets (e.g., species means vs. individuals), shape variation caused by different ecological pressures may be concentrated in specific parts of the structure instead of effecting the entire shape of it, as is the case for more simplified body parts as the legs.

In some spider species, where extreme trophic adaptation has translated into a dramatic modification of their chelicera, the musculature related to this structure runs further up to attach to apodemes on the posterior part of the prosoma (see Wood & Parkinson, 2019). Although this is not the general pattern in spiders (e.g., Lin et al., 2021), it would be reasonable to predict that shape variation in the chelicera would also translate into coordinated differences in the carapace. This prediction is partially confirmed by high values of integration of some of the LM configurations of the chelicera and carapace (Figure 3). However, our results show that species that are phenotypically similar across the cheliceral morphospace are not necessarily similar in the carapace morphospace. Interestingly, some of the most extreme morphologies observed in this morphospace corresponded to the step-shaped carapace species (*D. breviseta* and *D. macra*; Arnedo & Ribera, 1999), on the one hand, and to the flat-fang species on the other hand (*D. ramblae*; Figure 4d). The species with this flattened fang is mainly differentiated across PC2, which captures shape variation related to the relative width of the frontal part of the prosoma and the absence or presence of the step-shaped carapace in the lateral view (Figure 6b). Given the particular shape of this extreme modification in the chelicera, the more narrowed frontal part and the absence of a step-shaped carapace observed in this species can be interpreted as a result of a lack of need to accommodate large cheliceral musculature, suggesting that visible coordinated shape variation between the chelicera and the carapace would only be observed in extreme cases. However, a more detailed study of the link between the chelicera musculature

attachments and the carapace shape in the *Dysdera* species will be necessary to confirm or reject such a biomechanical explanation of the integration between both body structures.

The distribution of species across the morphospace corresponding to the subset combination of all the different LM configurations combined does not seem to be associated with any obvious ecological or biomechanical explanation. However, the lower intra-specific dispersion observed and the decrease in the overlap between species indicate that the phenotypic identity of each species is better defined when combining information from all LM configurations across different body structures. This observation is in line with the fact that the use of a greater number of phenotypic variables adds resolution to the description of morphological variation (Collyer et al., 2015). In this sense, then, the phenotypic space that each species occupies can be more properly delimited using whole-body information, rather than focusing on specific traits which, despite capturing the ecological and morphological particularities of each species, they are not as efficient for characterizing interspecific morphological differences.

4.3 | What body structures to study?

Adaptive evolution in response to habitat variation is a major driver of phenotypic variation in arachnids, including spiders (Gonçalves-Souza et al., 2014) and scorpions (Coelho et al., 2021). Our exploratory analysis of species with extreme ecological habits within Canarian *Dysdera* suggests that variation across different dimensions of the ecological niche may be reflected in variation patterns of distinct structures of the spider phenotype. In *Dysdera* species, it seems that the phenotypic variation of the chelicera is associated with trophic adaptations related to the predation on isopods (Řezáč et al., 2021; Toft & Macías-Hernández, 2021) and that adaptation to underground environments is markedly reflected in their leg morphology (Arnedo et al., 2007). However, the evolutionary pressures affecting the shape of different body parts in other spider groups could vary drastically, influencing biological inferences. For instance, some *Tetragnatha* Latreille (1804) spiders exhibit marked sexual dimorphism, where the male phenotype is not related to feeding specialization (Baba et al., 2018; Lesar & Unzicker, 1978; Makoto, 1987). Similarly, in some species of jumping spiders, sexual selection is involved in determining the morphology of the legs in males, as these are involved in courtship behavior (see Lai et al., 2021). Therefore, a good biological knowledge of the species at hand is required before selecting the views and structures to analyze and test the hypotheses to link them to any evolutionary adaptation. Nevertheless, we have shown that variation in trophic habits, in a group without apparent sexual selection pressures in the external phenotype, as is the case of the *Dysdera* spider species (Cooke, 1965), is readily captured by considering different LM configurations of the chelicera. In the same way, some habitat-related questions can be better answered with the analysis of the limbs. Finally, the global phenotypic space occupied by each species would be better

delimited by putting together different, and apparently not related structures to hoard the morphological differences that exist across species. For example, one may be interested in differentiating species regardless of the ecological meaning of phenotypic structures. In such a scenario, analyses of morphological variation become more efficient if considering all the characters that make these species different and examining how they differ across a global morphospace.

5 | CONCLUSIONS

Taken together, the multipart analyses conducted here indicate that a proper definition of the morphospace occupied by different species is optimized by combining information from different unrelated structures. However, our analyses also suggest that different ecological evolutionary pressures influence different parts of the phenotype of the *Dysdera* species from the Canary Islands in different directions. Although a formal, comprehensive analysis of ecomorphological evolution in this model system is still pending, different morphospaces seem associated with distinct ecological features, where species form different groupings and aggregations depending on the body structure analyzed and the ecological function for which it is relevant. We have shown that although all morphological LM configurations show some degree of integration, those related to the same morphological structure (chelicera, prosoma, and legs) exhibit significantly higher integration values, and are thus more strongly interdependent functionally and evolutionarily. The exploration undertaken here provides a guidance for future work focused on the study of morphological structures that seek to link different parts of a phenotype to the potential underlying ecological, biomechanical, social, or other pressures that have shaped them.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available on Github at https://github.com/EvoDysdera/Vitruvian_Dysdera.

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