

Review article**Morphometric Methods in the Taxonomy of Hydrophilidae and Helophoridae (Coleoptera): A Review of Current Approaches in Türkiye****Ayçin AKÜNAL** 

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Abstract: The families Hydrophilidae and Helophoridae are among the most important groups of water beetles, and the species within these families exhibit considerable morphological variation in both body parts and male reproductive organs. This review examined the suitability of traditional and geometric morphometric methods for identifying species in these families. Accordingly, traditional morphometric analyses supplemented by an examination of the hair arrangement (chaetotaxy) of the species at the larval stage, as well as an integrated approach incorporating landmark-based geometric morphometry and molecular data at the adult stage, are considered the preferred methodology. Although there are numerous studies in Türkiye that examine the systematics, ecological preferences, and extent of exposure to pollutants in the families Hydrophilidae and Helophoridae, studies that combine morphometric analyses with molecular methods are relatively rare. The studies reviewed suggest that chaetotaxy-assisted traditional morphometry may be the most effective approach during the larval stage, whereas landmark-based geometric morphometry appears to be more effective during the adult stage. This review suggests that studies in Türkiye employing these methods in conjunction with molecular data are extremely rare, and integrated taxonomic studies should be a priority for future research.

Keywords: Hydrophilidae, Helophoridae, morphometrics, traditional morphometrics, geometric morphometrics

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Introduction

Water beetle community structure responds closely to pollution, with reduced species richness, abundance and diversity in degraded sites, often signaling water quality deterioration before conventional physicochemical analyses detect it. Narrow-range diving beetles may indicate clean waters, while tolerant scavenger beetles may suggest organic enrichment (Al-Abadi and Hatem, 2025). Because such ecological responses are expressed across species and populations, the quantitative assessment of morphological characteristics is

important for reliable species identification, for distinguishing between populations, and determining their distribution in ecologically diverse habitats (Akünal and Aslan, 2017).

The family Hydrophilidae comprises over 3,000 species in 169 genera worldwide (Clarkson et al., 2018; Short, 2018; Short et al., 2021). Most species inhabit a variety of aquatic habitats, including ponds, streams, and riparian zones. While the larvae are predators, most adults feed on plant matter or decaying organic material (Clarkson et al., 2018). Along with Dytiscidae, Hydrophilidae represents one

of the most species-rich groups of aquatic beetles (Short, 2018).

Adult Hydrophilidae can be easily distinguished from other groups of aquatic insects by characteristics such as the almost complete absence of a break between the pronotum and the elytra, a non-granular structure of the pronotum and elytra, and a body covered with hydrofuge setae. Body length varies between 1.5 and 50 mm, depending on the taxon. The antennae have 7 to 9 segments, are short, and are attached in front of the eyes; the last three segments are spherical and bearing hydrofuge setae. The pronotum is generally convex, although some species have lateral slits. The markings on the elytra may vary depending on the genus. Tarsal morphology is taxonomically significant; tarsi typically comprise five segments; however, the meso-/metatarsus of some *Cymbiodyta* species and the protarsus of male *Berosus* species have four segments (Yilmaz, 2011; Topal, 2023). For species in the family Hydrophilidae, which exhibit quite diverse morphological characteristics, morphometric methods are necessary to quantitatively determine these differences.

Helophoridae is a monotypic family containing the single genus *Helophorus* Fabricius, 1775, which comprises approximately 200 species worldwide, divided into nine subgenera (Darılmaz, 2010). Adult occur in a range of habitats, from aquatic to semi-aquatic or terrestrial. *Helophorus* species are typically predatory in the larval stage and are found in terrestrial habitats (Minoshima and Watanabe, 2020). Members of the family Helophoridae can be easily distinguished from other aquatic members of the Hydrophiloidea by the five rows of longitudinal slits on their pronotum and their elongated body shape (Angus, 1992; Fikáček, 2009). In addition, they can be easily distinguished from other aquatic groups by their short scutellum, the rows of dots on their elytra, their five-segmented abdomen, and the fact that the last tarsal segment is longer than the third segment (Balfour-Browne, 1958).

Morphometry encompasses methods for recording and statistically analyzing form variation within and between organisms, including ontogenetic and evolutionary changes. Morphometric methods are used to define and compare the forms of organisms or specific structures. Morphometric research addresses diverse questions, including ontogenetic changes, sexual dimorphism, geographic variation, and ecological adaptation and habitats (Marcus, 1990). Morphology is an important source of

information in the natural sciences, materials science, and engineering. In all these fields, the shape of an object consists of two fundamental components: size and form. Therefore, morphometry is defined as the quantitative analysis of form and its covariances (MacLeod, 2017).

In traditional morphometry, the length and width of anatomical structures, distances between landmarks, and occasionally angles and ratios are measured (Rohlf and Marcus, 1993). Traditional morphometry has limitations in fully capturing form, leading to the development of geometric morphometric approaches. Traditional morphometry has undergone rapid change and has evolved into multivariate analyses. Geometric-morphometric analyses are based on a methodology that relies on landmarks and draws on imaging techniques (Özdemir and Can, 2023). By providing analytical and graphical tools, this method enables the identification of previously overlooked characteristics of organisms by decomposing variations into components that reflect differences across various spatial scales. In studies of geometric morphometrics, various methods – such as landmark-based techniques and elliptical Fourier analysis can be used to investigate the morphological characteristics of an organism. The landmark (Procrustes) method is considered the most theoretically sophisticated morphometric technique: it superimposes the landmark coordinates of specimens by estimating translation, rotation, and scaling parameters through least squares making shape differences visible. The resulting differences are then summarized at a new level using methods such as principal component analysis (PCA) (Özdemir and Can, 2023).

Organisms can be described using landmark-based geometric morphometry; intraspecific or interspecific differences can be analyzed using various methods such as canonical variance analysis and principal component analysis. This methodology is suitable for evaluating the morphological characteristics of morphologically important structures in invertebrates, such as the head, wings, and reproductive organs (Fidan and Şirin, 2024). In the family Hydrophilidae, where species are distinguished based on subtle morphological differences in body parts such as the pronotum and elytra, as well as in genital structures, the analysis of these characteristics using geometric morphometry can help both resolve taxonomic uncertainties and more reliably identify variations among populations.

The aim of this study is to evaluate the suitability of morphometric methods for identifying species in the families Hydrophilidae and Helophoridae, which is one of the most species-rich groups of aquatic beetles. The extensive morphological variation, particularly in male genitalia, renders morphometric analyses taxonomically valuable for this group. This review evaluates the contributions, applicability, and challenges of morphometric approaches to resolving identification problems within Hydrophilidae and Helophoridae, based on a comprehensive literature compilation. Although the number of international studies on the application of morphometric methods to the families Hydrophilidae and Helophoridae is increasing, there is no comprehensive review that systematically evaluates these studies and compares them with research findings from Türkiye. Therefore, this study aims to summarise existing literature from a critical perspective, evaluate the taxonomic applications of morphometric approaches, and provide guidance for future integrated morphometric and molecular studies.

Material and Methods

This study is a review aimed at evaluating the suitability of morphometric methods for identifying species of the Hydrophilidae and Helophoridae. The literature review was conducted using keywords such as morphometry, Hydrophilidae, Helophoridae, and morphometric analyses in Türkiye, supplemented by manual searches of relevant studies. The studies examined were evaluated comparatively regarding key aspects such as the morphometric method used (traditional/geometric), the body part analyzed (pronotum, elytra, genitalia, etc.), and the sample size. The analysis included studies published between 2008 and 2026 were identified primarily through the Web of Science and Scopus databases, with Google Scholar used as search engine.

Results

Morphometric Analyses by Developmental Stage

Studies based on morphometric methods have been conducted for various genera, in which the morphological characteristics of species in the family Hydrophilidae were measured. A review of these studies shows that the choice of method is largely determined by the sampling and measurement conditions of the developmental stage under investigation (larva or adult). In larval studies,

chaetotaxy and traditional morphometric analyses have generally been the preferred methods. As a first example supporting this trend, phylogenetic analyses were conducted on a comprehensive data matrix consisting of larval morphology and morphometry of species in the tribe Hydrobiusini, data on the arrangement of larval setae (larval chaetotaxy) to determine genus relationships within the tribe, and morphological characteristics of the adults. In addition, the arrangement of the head hairs in six genera within the tribe was described in detail; morphometric characters derived from the head capsule and mouthparts were also included in the analysis, and all characters were supported by detailed illustrations. An assessment of the diagnostic value of the characters revealed that larval characters generally exhibited a high degree of homoplasy, with chaetotaxic characters performing slightly worse than classical larval morphology, although both remained phylogenetically informative. Although larval chaetotaxy is used to understand evolutionary relationships, it is not sufficient on its own; corroborating it with morphological and molecular data helps to produce more reliable results (Archangelsky et al., 2021).

A similar approach was taken when comparing taxa adapted to different habitats. In another study, comparative analyses of the tribes Coelostomatini and *Sphaeridiini* were conducted to identify the morphological differences resulting from their adaptation to different habitats. According to the findings, the larvae of the Coelostomatini exhibited various terrestrial and aquatic morphotypes due to their adaptation to different habitats. The somatic morphology of *Dactylosternum* larvae showed similarities to larvae that live in decaying organic matter. In addition, the first detailed description of the hairiness of larvae of species in the genus *Sphaeridium* was presented; this study compared variations in hairiness as well as in the morphometry of the larvae's head structures and appendages (Rodriguez et al., 2025).

In another study that examined both the arrangement of larval hairs and morphometry, the egg sacs, three larval stages, and pupae of three species (*T. collaris*, *T. parananus*, and *T. scutellaris*) of the subgenus *Strepitornus*, which belongs to the genus *Tropisternus* (Hydrophilidae), were examined in detail concerning their morphometry and hair arrangement (chaetotaxy). Morphometric measurements and

chaetotaxic counts showed that the morphometric values of the species were largely similar; however, characteristics such as the width of the head capsule, antennomere proportions, the relationships of the maxillary segments, and the number of leg hairs were reliably useful for distinguishing between larval stages (Torres et al., 2008).

In another study examining various developmental processes, the arrangement of primary and secondary hair and the morphometric characteristics of the head capsule and head appendages were determined for three larval stages of *Dactylosternum cacti* and *Phaenonotum exstriatum*, both members of the tribe Coelostomatini, which belongs to the subfamily Sphaeridiinae. It was concluded that the arrangement of the bristles provides insight into the distinction between the genus and species levels within the Coelostomatini and can contribute to phylogenetic studies (Archangelsky et al., 2016).

The arrangement of primary and secondary setae, as well as the morphometric characteristics of the head capsule and head appendages of first- and third-instar larvae of the terrestrial species *Oosternum costatum* (Sphaeridiinae: Megasternini) from the family Hydrophilidae, were examined in detail for the first time. The sensilla (setae and pores) on the head capsule, antennae, mandibles, maxillae, and labium were marked according to the homology criterion and compared with the basic chaetotaxy scheme of the Hydrophilidae. It has been reported that the larvae of *O. costatum* share a considerable number of morphological and chaetotaxic characteristics with other known larvae of the tribe Megasternini (*Cercyon*, *Armostus*, *Motonerus*, *Cryptopleurum*, and *Pelosoma*); these shared characteristics strongly support the monophyly of the Megasternini (Archangelsky, 2018).

The use of chaetotaxy in species identification has been reported, as larvae of the genus *Enochrus* exhibits many structural characteristics that make it easy to distinguish them from other genera within the Enochrinae. Furthermore, despite their great similarity, larvae of different subgenera can be distinguished from one another based on various morphological characteristics and the arrangement of their setae. However, some characteristics vary even within the same subgenus. This underscores how important it is to identify new larvae even within known genera (Archangelsky, 2023).

The simpler and more reliable identification of landmarks in adult individuals enables the integrated

use of geometric morphometry and molecular data. A prime example of this is the integrated approach to defining species boundaries within the rare *Enochrus falcarius* complex, which inhabits predominantly saline waters in the Mediterranean region. First, hypotheses regarding the candidate species were formulated based on phylogenetic analyses and biogeographic data. Subsequently, the phylogenetic differentiation among the candidate species was assessed using the following methods: identification of molecular clusters, morphometry (both body morphometry and analysis of the pronotum contour), and estimates of ecological niche similarity. The morphometric analyses were based on 26 quantitative measurements of 26 key parts of the exoskeleton, which were taken from 84 individuals (54 males and 30 females). The results show that, despite a high degree of molecular differentiation between the species, the high morphological similarity is consistent with the previous classification of *E. falcarius* as a single species. Extreme environmental conditions, such as saline water, can promote morphological similarity among different lineages and thus explain the high degree of similarity observed in morphological analyses (Arribas et al., 2013).

As part of their geometric morphometric analyses, Kang et al. (2020) examined a total of 86 specimens of *Hydrochara affinis*, which had been both reared under laboratory conditions and collected from natural populations. To minimize measurement errors that might arise during the data collection process, the ventral side of each specimen was photographed three times, independently of one another. The resulting images provided the necessary dataset for analyzing the variation in the ventral body shape of *H. affinis* using a landmark-based and procrustes superimposition to geometric morphometrics. The study showed that geometric morphometry can be an effective tool for assessing the role of both environmental factors and genetic components in shaping morphological variation.

Hydrochara populations in Croatia were examined using an integrative approach that combined mitochondrial DNA data, morphometric analyses, and the morphology of male reproductive organs to investigate the cryptic genetic diversity of species in the genus *Hydrochara* in the floodplains of southeastern Europe. Morphometric analyses revealed a high degree of similarity between *Hydrochara dichroma* and *H. caraboides*, with no significant differences in external body size.

Examination of the male reproductive organs, however, revealed an intermediate aedeagus morphology with transitional characteristics between *H. caraboides* and *H. dichroma*. By combining molecular and morphological data, the study provides new insights into the diversity of *Hydrochara* lineages in floodplain ecosystems in Southeast Europe and underscores the importance of integrative approaches for clarifying species boundaries and conserving freshwater biodiversity (Turić et al., 2026).

Species in the family Helophoridae exhibit a high degree of variation in both male genitalia and body parts. Nevertheless, there are only a few studies that focus on classical or geometric morphometric analyses of species in the genus *Helophorus*. In this study, which also examined fossil specimens of relatives of this genus, a total of 92 morphological characteristics of larvae and adult specimens of *Helophorus* and other aquatic hydrophiloids were analyzed (Fikáček et al., 2012).

A review of the studies summarized above shows that traditional morphometric analyses are conducted in conjunction with chaetotaxy, particularly in the larval stages of various genera within the family Hydrophilidae. This demonstrates that these datasets can be used for species-level identification and make a valuable contribution to phylogenetic analyses. In the taxonomy of adult insects in this family, geometric morphometrics has been used to study the body shape of adults and the shape of male reproductive organs. Geometric morphometrics is more commonly applied to adult specimens, as it is often more difficult to define homologous landmarks on larval structures; however, it can also be applied to larvae when suitable landmarks or outlines are available.

Examples of morphometric analyses of families of aquatic beetles in Türkiye

In Türkiye, there are numerous regional studies on the systematics, ecological preferences, and extent of the effects of environmental pollution on the families Hydrophilidae and Helophoridae in their habitats (Akünel, 2024; Aydın et al., 2021; Aydoğan et al., 2022; Bektaş and Tepe, 2024; Caf et al., 2020; Polat et al., 2021; Polat, 2023; Taşar, 2023; Zeybek et al., 2024). However, studies on molecular analyses, morphometric analyses, or integrated taxonomic approaches to species identification for these aquatic groups are quite limited.

A study in Türkiye, one of the few combining morphometric and molecular methods, compared male specimens of *Hydrochara caraboides* (Coleoptera:

Hydrophilidae) from a natural wetland and an urban wetland in Erzurum using both data types. Ten specimens were collected from each area, and characteristics such as body length, antennae, maxillary palps, third pair of legs, wing covers, and aedeagus were measured to the nearest millimeter using a stereomicroscope. In addition, the species identification of the specimens was confirmed molecularly by amplifying the gene region of the mitochondrial cytochrome c oxidase subunit 1 using PCR. The results showed that all measured body structures of the individuals collected in the urban wetland were statistically smaller than those from the natural area (Bektaş, 2021).

Among the species of the family Helophoridae, there are significant differences in male reproductive organs and morphology. In a further study of representatives of this family, sexual dimorphism and structural anomalies were examined through morphometric analyses of Helophoridae specimens collected between April and October 2021 in various wetlands in Erzurum Province. Measurements of the species *Helophorus aquaticus* (Coleoptera) revealed sex-specific differences between male and female individuals ranging from 0.1 to 0.4 mm in body length, 0.1 to 0.3 mm in body width, and 0.1 to 0.3 mm in leg length. Geometric and classical morphometric methods are important tools in systematic and evolutionary studies due to their low cost, speed, and informative value, and it is believed that the combined analysis of morphometric data and structural anomalies will contribute significantly to the determination of the taxonomic hierarchies of aquatic insects and water beetles (Bektaş, 2022).

Traditional morphometric measurements, particularly those aimed at determining the influence of environmental factors on body size and shape, have so far been studied only to a limited extent in Türkiye, primarily in species of the family Helophoridae, which was formerly a subfamily of the family Hydrophilidae and was later elevated to a family in its own right. In a study aimed at determining the changes in the morphology of aquatic insects caused by environmental factors, the influence of differences in elevation on variations in body measurements and morphological characteristics of the *Helophorus aquaticus* was investigated. Specimens collected between 2007 and 2021 from various regions of Türkiye, from areas with different ecological characteristics, and from museums were photographed, and their coloration, body length, the structure of their aedeagus, and leg length were recorded. The results showed that different variants of the same species can live in the same habitat and that the size of the insects increases with altitude; however, no statistically significant correlation was found between wing width and leg length (Polat, 2023).

Conclusion

When identifying and systematically studying the families Hydrophilidae and Helophoridae, the preference for

morphometric methods appears to vary depending on the developmental stage being studied. In the studies reviewed, traditional morphometry supported by chaetotaxy was generally used for the larval stage, whereas landmark-based geometric morphometrics, often combined with molecular data, was more commonly applied to the adult stage. This is likely due not only to methodological preference but also to the different measurement methods used at various life stages. During the larval stage, the low degree of sclerotisation and changes in body shape make it difficult to clearly define landmarks, which limits the applicability of geometric morphometrics during this period. In adults, however, the completion of development and the resulting reproducible marking of landmarks provide useful data for morphometric analyses. Another notable finding from studies of adults is the reporting of cases in which morphological similarity is preserved despite a high degree of molecular differentiation.

This suggests that morphometric analyses can provide valuable information on their own, but that molecular data can serve as complementary evidence and increase the reliability of species-level conclusions, particularly in groups that include cryptic or semicryptic species complexes. Furthermore, the review emphasises the need for a holistic approach in future studies, one that considers measurements of morphological characteristics along with differences in male reproductive organs and supports these findings with molecular data to prioritise methodological decisions regarding the delimitation of species within the family Hydrophilidae.

Morphometric studies of Hydrophilidae and Helophoridae in Türkiye follow three main approaches: determining the influence of environmental factors (altitude, temperature) on body size using classical measurements; identifying sexual dimorphism and structural anomalies; and examining form-based taxonomic distinctions using landmark-based geometric morphometry. The combined use of these methods not only increases the reliability of species identification but also contributes to a more comprehensive understanding of the ecological adaptation mechanisms of aquatic insects. Numerous studies worldwide use morphometric analyses for various genera of aquatic beetles. In Türkiye, however, studies that use morphometric methods to contribute to molecular taxonomy, particularly for the families Hydrophilidae and Helophoridae are extremely rare. Studies limited to classical morphometry may be less effective at discriminating closely related taxa than approaches that incorporate geometric morphometric or genetic data. Addressing these shortcomings is of crucial importance for these highly heterogeneous groups, particularly for identifying cryptic species, determining the effects of environmental parameters on morphology, and understanding the relationships among species.

Ethical Approval

No need to ethical approval for this study.

Conflicts of Interest

The author declares that she has no conflict of interest.

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