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The problem of 'shadow species' as illustrated with the taxonomic hotchpotch *Cyphoderia ampulla* (Rhizaria: Cyphoderiidae)

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ABSTRACT

Accurate species delimitation based on an integrative approach is indispensable for biodiversity evaluation. Accordingly, species that were formerly only described morphologically often need to be split into several evolutionary independent units. However, taxonomic actions often lag behind, even when the required data are already available. As a result, invalid species names are carried over the years, with negative implications on ecology, biogeography, and conservation; we designate these entities as 'shadow species'. This is particularly common in protists, due to the lack of specialized taxonomists and the difficulties of working with microscopic organisms. Here, we resolve the case of the testate amoeba shadow species *Cyphoderia ampulla* (Rhizaria: Cercozoa: Euglyphida: Cyphoderiidae), a known polyphyletic taxon. Purposely, we integrated the current ecological, genetic, and morphological data on the family Cyphoderiidae with its described evolutionary history. Subsequently, we took the required taxonomic actions to resolve the taxonomy of the family, erecting four new genera (***Psammoderia gen nov., Knarr gen. nov., Ichthyosquama gen. nov., and Oleiformis gen. nov.***), emending *Cyphoderia* and describing four new species (***Ichthyosquama sanabriensis* sp. nov.**, ***Ichthyosquama catoirensis* sp. nov.**, ***Ichthyosquama loricaria* sp. nov.**, and ***Oleiformis carmelae* sp. nov.**). Finally, we discuss the extension and relevance of the 'shadow species' issue in eukaryotic taxa, and differentiate it from species complex concept.

Keywords: species concepts; species delineation; taxa; taxonomy

INTRODUCTION

Biodiversity is composed of species, that is, populations that share a unique evolutionary history that separates them from other closely related populations (Wiley 1978). Delimiting the boundaries between these population units requires characterising their past and future evolutionary trajectories, and comparing the most closely-related ones. For this purpose, a theoretical background has been developed [i.e. 'species concepts' (De Queiroz 2007)], which provides the conceptual tools to characterize these limits. Once these boundaries have been identified these biological units can be described as species and given a unique scientific name, following an international code of nomenclature, International Code of Zoological Nomenclature (ICZN) (zoology) or International Code of Nomenclature (ICN) (botany), depending on the research tradition to which organisms belong (Lahr *et al.* 2012).

The theory developed about these species concepts was linked with the emergence of new technologies that allowed the study of ecology, morphology, or physiology from a new perspective. This was the case with the inclusion of molecular tools in systematics, which revealed the widespread existence of cryptic species that could not be delimited with morphology alone (Taylor *et al.* 2000, Bickford *et al.* 2007, Struck and Cerca 2019), or convergent evolution between distantly-related organisms (Wilhelmi and Krenn 2012, Meyer *et al.* 2013, Partha *et al.* 2017); morphology alone does not suffice to delimit species. In this context, it happens that species that were described before the application of molecular tools frequently need to be divided into several new taxonomic units. Examples are widespread in animals and plants; a large number of the species described in Europe in the past decade are the product of splitting larger species complexes that appeared arguably homogeneous (Recuero

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et al. 2012, Ghielmi *et al.* 2016, Sangster *et al.* 2016, Criado Ruiz *et al.* 2021). In smaller organisms with fewer morphological features, such as protists, this case should be, at least, equally widespread (e.g. Kosakyan *et al.* 2012, Hayward *et al.* 2021).

In spite of their similar morphology, describing and naming these 'hidden' species is essential, simply because species constitute the basic components of biodiversity. In vertebrates, new species can motivate public opinion towards conservation actions that can target both species and ecosystems (Oliva-Paterna *et al.* 2006); this is much less common in small organisms such as protists (but see Cotterill *et al.* 2013). These actions are nevertheless justified because protists with similar morphologies may have different niches and thus have different functions in the ecosystem (Singer *et al.* 2019). A good knowledge of protist diversity is key for understanding crucial ecological processes as well as system resilience (Fetzer *et al.* 2015), phylogeographical (Singer *et al.* 2019, Pinseel *et al.* 2020) and macroevolutionary patterns (González-Miguéns *et al.* 2022), and, on a more applied scope, bioindication and ecosystem biomonitoring (Pawlowski *et al.* 2016).

However, in practice, the species characterization and description are often lagging, if not postponed forever. The decreasing number of taxonomists due to limited funding and job opportunities has been widely discussed (Engel *et al.* 2021) and certainly plays an important role in this delay. In addition, difficulties inherent to protist taxonomy such as the scarcity of characteristic traits and the impossibility of culturing most species are additional burdens to protist taxonomists. For these reasons, even though the data that can be used to characterize the independence of each species from other closely related taxa exist, the whole process leading to the description and scientific naming of individual species is often not fulfilled. As a result, these taxonomic units with an informal status (named here 'shadow species'), coexist with the same scientific name in a limbo that lasts sometimes for decades, sometimes more. These shadow species typically have wide ecological tolerance, cosmopolitan distributions, or a variable morphology because they are in fact species conglomerates; sometimes they are not even monophyletic. Keeping these shadow species in the taxonomic literature leads to an underestimation of biodiversity, and thus to misinterpreting ecological or evolutionary processes.

Here, we illustrate the problem of shadow species with the case of the protist *Cyphoderia ampulla* (Ehrenberg, 1840) (Cercozoa: Euglyphida). This amoeboid organism is characterized by a self-secreted shell (or test) with a bota bag shape, reinforced with silica scales. It has been claimed to be distributed worldwide in ecosystems as disparate as *Sphagnum* peatlands, rivers, coastal sediments, and brackish waters (Ogden and Hedley 1980). A detailed morphological study on *C. ampulla* populations showed the existence of distinct morphologies in different populations, which suggested the existence of several potential species classified under the same name (Todorov *et al.* 2009). This hypothesis was later confirmed by barcoding with nuclear (Heger *et al.* 2010) and mitochondrial (Heger *et al.* 2011) molecular markers. Later, a study demonstrated further the independent evolutionary histories of these populations, with a special emphasis on ecological transitions from marine to freshwater environments as the main drivers for the evolutionary history of the group (González-Miguéns *et al.* 2022). Eventually, these transitions were accompanied with an increase in size in the freshwater forms, together with a general homogenization of morphology, most likely by evolutionary convergence. The demonstration of the independent evolutionary histories of these lineages is a prerequisite for considering them as different species. *Cyphoderia ampulla* is therefore a shadow species at this moment, since the data and evidence necessary for the description of the species are currently available, but no taxonomic action has yet been taken.

In this work, we resolved the taxonomic situation of the shadow species *C. ampulla* by reviewing the existing taxonomy of genus *Cyphoderia*, and integrating the previously published data of morphology, and ecology, as well as sequences of both the nuclear gene coding for the RNA of the small subunit of the ribosome (*SSU*) and mitochondrial cytochrome oxidase subunit I (*COI*). We justify our taxonomic decisions (species description and new genera erection) based on this integrative approach, thus providing an example of how shadow species problems can be handled in all eukaryotes no matter the size.

MATERIAL AND METHODS

Molecular phylogeny

The molecular *COI* and *SSU* data used here come from Heger *et al.* (2010, 2011) and González-Miguéns *et al.* (2022), and *COI* and *SSU* phylogenies were the same as in González-Miguéns *et al.* (2022). Hence, based on these phylogenies we name 10 and 11 different clades for *SSU* and *COI* trees, respectively (described in the molecular phylogeny results). Then we calculated, for *COI* and *SSU* respectively, genetic distance between these groups (as defined in the phylogenetic tree), measuring the number of base substitutions per site from averaging over all *COI* and *SSU* sequence pairs between each clade described above using the Tamura 3-parameter substitution model implemented in the software MEGA v.10.1.8 (Kumar *et al.* 2018). Then, we took the pair of groups that diverged the least genetically but that had unambiguously different morphologies, *Cyphoderia trochus* and *Cyphoderia amphoralis*, and set this distance as the minimum to separate two species.

Morphometric analyses

The measurements of 689 cells shells for morphological analyses were taken from Todorov *et al.* (2009) and González-Miguéns *et al.* (2022) (the data are available in MorphoBank: http://morphobank.org/permalink/?P4082). We used the mitochondrial clades described in González-Miguéns *et al.* (2022) as a reference on which to perform a linear discriminant analysis (LDA), using the variables 'shell length', 'shell width', 'large pseudostome axis', and 'length-width ratio' (after Todorov *et al.* 2009 and González-Miguéns *et al.* 2022) with the package MASS (Venables & Ripley 2002). Morphological analyses were performed using R software v.4.1.2 (R Core Team 2021) implemented in RStudio v.4.1.2 (Haque *et al.* 2017). The outliers were retrieved with the package stats (R Core Team 2021). Finally, the package ggplot2 (Wickham 2016) was used to generate a scatterplot.

Organism identifications and taxonomic decisions

An integrative taxonomic approach was carried out to perform the taxonomic decisions on the *Cyphoderia* taxa (González-Miguéns *et al.* 2022). This approach included phylogenies with two markers (*COI* and *SSU*), a morphometric analysis, and

information about the type of environment where the specimens were isolated (marine supralittoral vs. freshwater). Phylogenetic clades were considered as species when they were recovered in both *COI* and *SSU* trees and when morphology and ecology were congruent (Kosakyan *et al.* 2016, Inoue *et al.* 2020, Lara *et al.* 2020, González-Miguéns *et al.* 2022). These newly defined species were compared with the existing relevant literature and in particular with the original illustrations, as they constitute the type material for Euglyphida species.

We deposited metallized stubs as type and paratype for the new species described material in the collection of the Royal Botanical Garden under the codes MA-Amoeba 11261-11265. These same stubs were used to obtain the scanning electron micrographs illustrated in González-Miguéns *et al.* (2022). The type material of the species from Rhodopes, Bulgaria, is preserved as a permanent microscopic preparation in Canada balsam in the National Museum of Natural History in Sofia by the Distributed System of Scientific Collections (DiSSCo) project under the name BG-IBER-INV-000016458. The rules and recommendations of the ICZN were applied to take the taxonomic decisions, which apply to testate amoebae (Lahr *et al.* 2012).

In order to facilitate the distinction between the genera of Cyphoderiidae, a dichotomous key to the family has been made. This key is composed of distinctive characters of each genus, such as habitat, arrangement and shape of scales, shell outline, size of shell, and shape of the shell opening. In addition, shell drawings, scale arrangement under scanning electron microscopy (SEM) and shell outline under light microscopy are provided for new genera (Fig. 3). Along with this, in order to visualize the morphological variation found within a single species, we prepared a figure that illustrates the variability within one of the new species described (*Oleiformis carmelae,* Supporting Information, Fig. S1).

Literature search

Exhaustive research was carried out compiling all the original species descriptions of genus *Cyphoderia* from the available published research. A synonymic list, together with references to all the original manuscripts, can be found in the Supporting Information, Appendix S1. From each publication the following information was obtained, where possible: data on the precise geographic location from where species were described, description of the original ecosystem, shell morphology, scale shape, and organization and images/drawings of the type specimens.

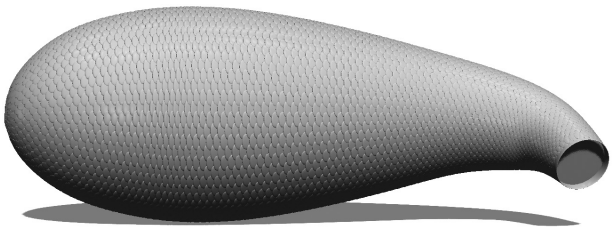
3D models

The 3D models of two shells, from the clades B and C, were built using Blender v.2.93.4, http://blender.org (Community 2021) and are available in the Sketchfab links https://skfb.ly/oAuQp and https://skfb.ly/oAuQC for Model 1 and Model 2 respectively. The protocols used for 3D model design can be found in the Supporting Information, Appendix S2. The aim of building these 3D images is to facilitate the interpretation of shell morphology for both the scientific community and the general public as educational material.

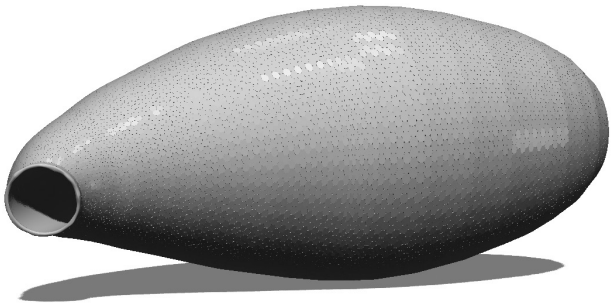
RESULTS

Molecular phylogeny

According to the *COI* and *SSU* phylogenies of González-Miguéns *et al.* (2022), the genus *Cyphoderia* can be split into five different monophyletic clades: clade A (containing only species assigned to *C. ampulla*), clade B (the *Cyphoderia major* clade), clade C (the *C. amphoralis*, *C. ampulla*, and *C. trochus* clades), clade D (the *Cyphoderia compressa* clade), clade E (*Cyphoderia littoralis*, with only *SSU* data), and clade F (*Cyphoderia* cf. *compressa*, with only *COI* data; this clade lacks morphological data and has been discarded it from further analyses). Clade E occupies a basal position with respect to A, B, C, and D, but its exact position in the tree still remains to be determined with more molecular data and more taxa. All clades are robustly supported both in Bayesian analyses (almost all posterior probabilities = 1) and Maximum Likelihood (bootstraps > 95%) (Fig. 1).



Model 1. 3D representation of ***Ichthyosquama catoirensis* sp. nov.**



Model 2. 3D representation of ***Oleiformis carmelae* sp. nov.**

Each of these clades could also be divided into subclades, based on the shell morphology and the well supported nodes in both in Bayesian and Maximum Likelihood phylogenetic analyses. These subclades sometimes corresponded to species already described based on their morphology; for instance, subclades C1 and C5 corresponded, respectively, to the species *C. amphoralis* and *C. trochus*. Clade A was composed of three subclades (A1–A3), clade B by two subclades (B1 and B2), and clade C by five subclades (C1–C5). Genetic distances between subclades reached 0.0473 (*COI*) and 0.0061 (*SSU*) within clade A, 0.0681 (*COI*) within clade B, and 0.0889 (*COI*) and 0.0233 (*SSU*) in C, respectively (Supporting Information, Table S1).

Morphometrics and morphology

We presented the results of a linear discriminant analysis (LDA) in three scatterplots with the linear discriminant functions 1 (LD1) and 2 (LD2) scores for all subclades of genus *Cyphoderia*, and for subclades C1–C5 and A1–A3, respectively (Fig. 2A, B). This analysis could delimit part of the different groups (i.e. clades and subclades) in the genus *Cyphoderia*. The percentages of different linear discriminant axes were 59.95% for LD1 and 98.06% for LD2, respectively (Table 1). LDA classified 100% of individuals belonging to clades D and E correctly. Within clade

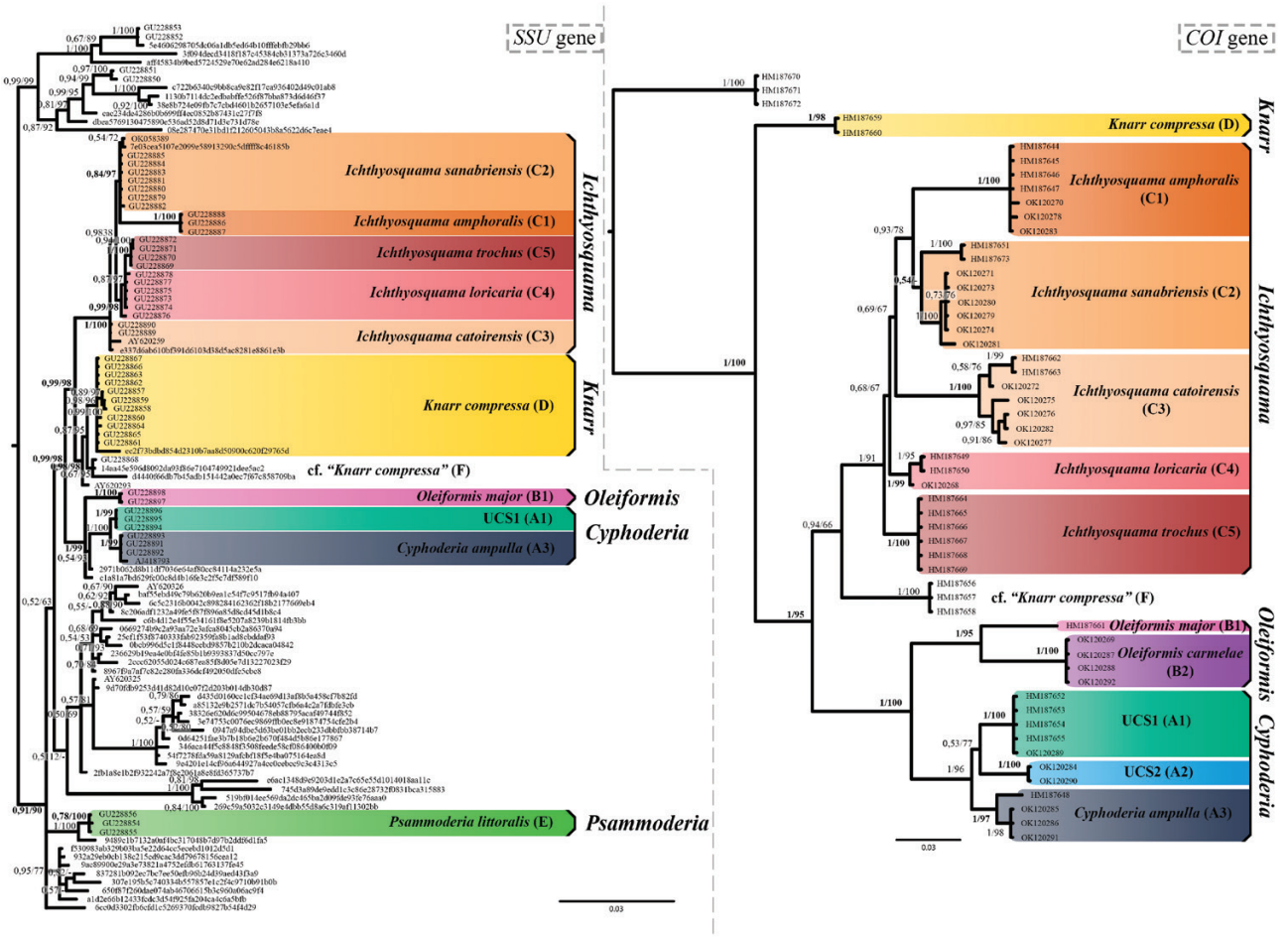


Figure 1. Bayesian phylogenetic tree based on *SSU* and *COI* sequences of the family Cyphoderiidae. At each node posterior probability values (Bayesian analysis) and bootstrap values (Maximum Likelihood analysis) are represented. The colours represent the molecular clades. GenBank accession codes are given on the tips. Species names correspond to the taxonomic assignation of the organisms based on morphology for each lineage.

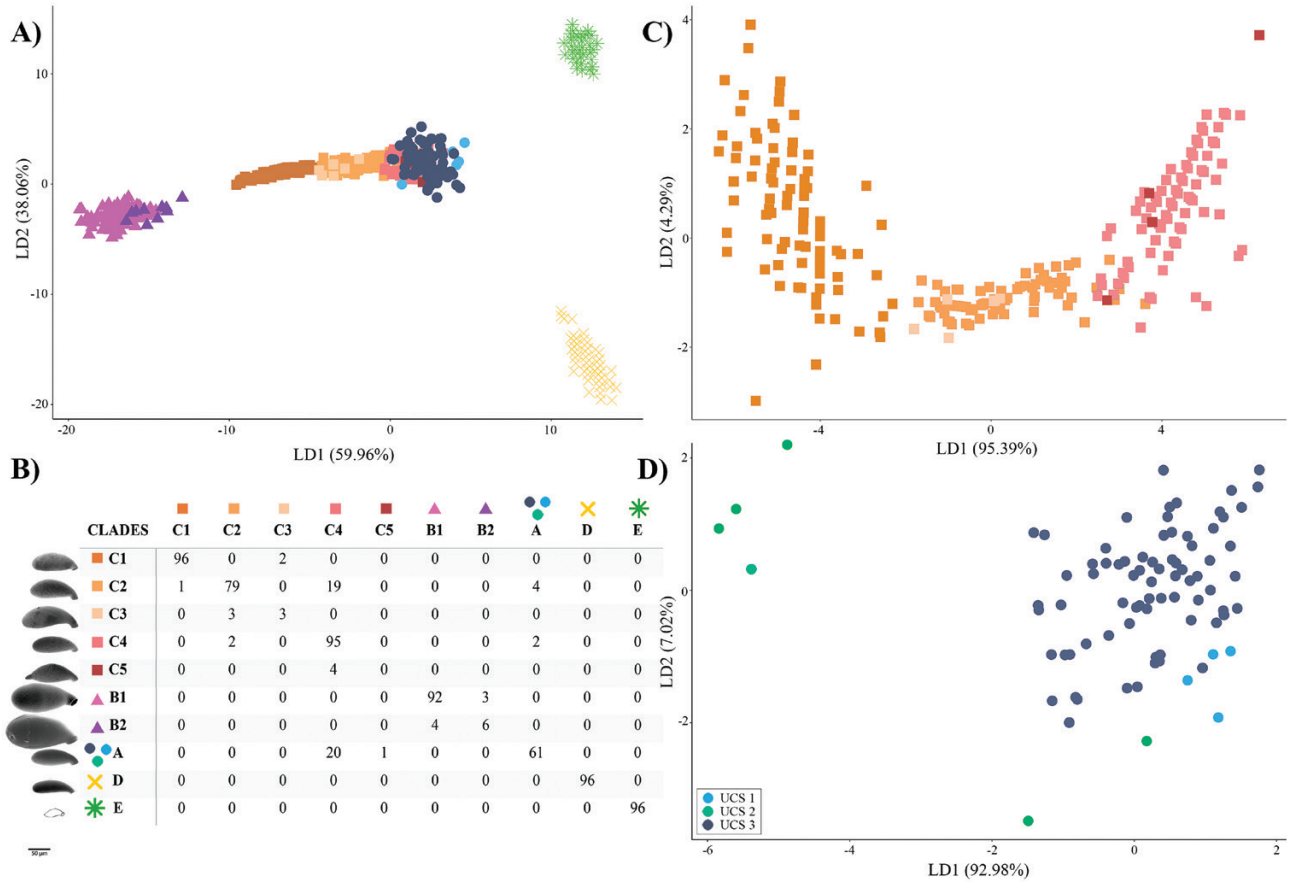
B, the subclades B1 and B2 showed an important overlapping zone between their shell morphospace. Likewise, clades A and C do overlap, as well as subclades within C to some extent (Fig. 2B; Table 2).

In the case of the subclades that compose the clade A, the percentages for LD1 and LD2 were 92.98% and 7.02%, respectively (Table 1). The analysis allowed an efficient classification of morphologies into the different subclades (93.02% probability of discrimination; Fig. 2B, D; Table 3) when only these subclades were taken into account; however, a higher number of shells (especially from A1 and A2) would be needed to corroborate this result. Within subclades C1–C5, the percentages of different linear discriminant axes were 95.39% for LD1 and 4.29% for LD2, respectively (Table 1). In all clades, shells were classified more accurately within the different mitochondrial subclades (93.84% probability of discrimination; Fig. 2B, C; Table 4) when analyses are performed within single groups (i.e. separately from other clades).

DISCUSSION

The problem of the 'shadow species'

Species delimitation and characterization is a complex task, and there is a need for a compromise between the theory and practical aspects. A widely used definition of species is 'a single lineage of ancestral descendant populations of organisms which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate' (Wiley 1978). To trace these evolutionary histories there is a whole body of literature on 'species concepts' that can be used as operational tools for discovering, delimiting, and diagnosing species (De Queiroz 2007). However, in protists, and especially in uncultured unicellular organisms, many of these concepts are difficult to apply as they require large and independent data on ecology, morphology, and genomics to trace evolutionary histories; obtaining these data is, presently, nearly impossible. For that purpose, informal concepts have been created for microorganisms such as the 'biospecies' applied to Myxomycetes (i.e. Feng *et al.* 2016). The 'biospecies' is opposed to the 'morphospecies', as delimitation is achieved on genetic criteria rather than on morphology, and an organism do not receive a Linnaean name if it cannot be differentiated from their closest relatives based on visible traits. The concept of 'visible traits' depends on the technology employed for the observation, and also on the beholder; thus, it remains rather subjective. We prefer adhering to Wiley's strict definition, though acknowledging the existence of species morphologically difficult or impossible to discriminate from each other. We therefore encourage naming 'cryptic'



CLADES	C1	C2	C3	C4	C5	B1	B2	A	D	E
C1	96	0	2	0	0	0	0	0	0	0
C2	1	79	0	19	0	0	0	4	0	0
C3	0	3	3	0	0	0	0	0	0	0
C4	0	2	0	95	0	0	0	2	0	0
C5	0	0	0	4	0	0	0	0	0	0
B1	0	0	0	0	0	92	3	0	0	0
B2	0	0	0	0	0	4	6	0	0	0
A	0	0	0	20	1	0	0	61	0	0
D	0	0	0	0	0	0	0	0	96	0
E	0	0	0	0	0	0	0	0	0	96

Figure 2. A, Linear discriminant analysis of four continuous morphological traits. B, Number of shells classified into their own clade under the linear discriminant analysis. C, Linear discriminant analysis of four continuous morphological traits of clade C. D, Linear discriminant analysis of four continuous morphological traits of clade A.

Table 1. Linear discriminant axes calculated using the linear discriminant analysis (LDA) model for all genera (*Cyphoderia*, *Ichthyosquama*, *Knarr*, *Oleiformis* and *Psammoderia*), and for the ones that overlap (*Cyphoderia* and *Ichthyosquama*).

	Linear Discriminant Axes			
	LD1	LD2	LD3	LD4
All genera	0.5995	0.9806	0.0169	0.0029
Ichthyosquama	0.9539	0.0429	0.002	0.0012
Cyphoderia	0.9298	0.0702		

species that can be separated by molecular or ecology criteria, and giving them a Linnean name, even though their morphology overlaps partially or totally with other species (see Lara *et al.* 2020). Linnean names are the only valid names universally accepted to designate species. The co-existence of several independent taxonomic units sharing the same Linnean name in the literature (i.e. the shadow species) prevents the replication of experiments, biodiversity assessments at all levels, etc.

It must be noticed that shadow species should not be confused with species complexes. The latter concept consists in closely related taxa that were hidden under the same Linnean name, that exhibit similar characteristics that making delimitation difficult , but that have been eventually formally described as two or more species. We apply the concept of shadow species to cases where there is enough evidence to allow taxonomic descriptions and assignation of Linnean names, but this procedure was not applied. Shadow species should not be confused with Unconfirmed Candidate Species (UCS), as the latter refers to lineages for which more information is needed to confirm their specific status (Vieites *et al.* 2009). The coexistence of these three concepts, shadow species, species complexes, and UCS is however often influenced to some extent by the researcher's subjectivity in the process of species description.

Examples of shadow species are present in all of the eukaryotic tree, and not only in microbial-sized organisms. The fire salamander *Salamandra salamandra* Linnaeus, 1758, includes several subspecies that are well characterized molecularly or morphologically, and which could arguably be given species status (Burgon *et al.* 2021). Such taxonomic actions would have positive consequences on conservation, as well as on taking actions to prevent the spread of the parasitic fungus *Batrachochytridium salamandrivorans* (Martel *et al.* 2013) which is lethal to salamanders (Burgon *et al.* 2021). There are more potential cases of shadow species in iconic species such as the killer whale *Orcinus orca* (Linnaeus, 1758) (Morin *et al.*, 2010) or the buff-tailed bumblebee *Bombus terrestris* (Linnaeus, 1758) (Rasmont *et al.* 2008, Lecocq *et al.* 2016). The description of the different species that compose these shadow species usually takes several years, or even decades, as in the case of the European earwig *Forficula auricularia* Linnaeus, 1758. This shadow species was

Table 2. Number of well-classified shells in their own clade and probability of discrimination with respect to the total number of shells per clade.

Clades	C1	C2	C3	C4	C5	B1	B2	A	D	E	Total Shells	Probability Of Discrimination (%)
C1	96	0	2	0	0	0	0	0	0	0	98	97.96
C2	1	79	0	19	0	0	0	4	0	0	103	76.70
C3	0	3	3	0	0	0	0	0	0	0	6	50
C4	0	2	0	95	0	0	0	2	0	0	99	95.96
C5	0	0	0	4	0	0	0	0	0	0	4	0
B1	0	0	0	0	0	92	3	0	0	0	95	96.84
B2	0	0	0	0	0	4	6	0	0	0	10	60
A	0	0	0	20	1	0	0	61	0	0	82	74.39
D	0	0	0	0	0	0	0	0	96	0	96	100
E	0	0	0	0	0	0	0	0	0	96	96	100
All Clades											689	90.57

Table 3. Number of clade A well-classified shells in their own clade using linear discriminant analysis (LDA) and probability of discrimination with respect to the total number of shells per clade.

Clades	A1	A2	A3	Total Shells	Probability Of Discrimination (%)
A1	0	0	4	4	100
A2	0	4	2	6	66.67
A3	0	0	76	76	100
All Clades				86	93.02

Table 4. Number of clade C well-classified shells in their own clade using linear discriminant analysis (LDA) and probability of discrimination with respect to the total number of shells per clade.

Clades	C1	C2	C3	C4	C5	Total Shells	Probability Of Discrimination (%)
C1	96	1	2	0	0	98	97.96
C2	0	83	0	4	0	87	95.40
C3	1	6	0	0	0	7	50
C4	0	1	0	95	0	96	85.71
C5	0	0	0	4	0	4	0
All Clades						292	93.84

characterized as a cryptic complex of at least two taxonomic units, demonstrated with postzygotic isolation and several molecular markers (Wirth *et al.* 1998); but their taxonomic status was not established until more than 20 years later (González-Miguéns *et al.* 2020). In microbial-sized eukaryotes, the shadow species issue is naturally even more frequent given the small size of the protist taxonomist's community. Examples are manifold; the testate amoeba *Euglypha rotunda* Wailes & Penard, 1911 is known to be a shadow species since the past decade based on molecular and morphological data (Wylezich *et al.* 2002, Lara *et al.* 2007) but still appears as a single taxonomic unit in recent ecological studies (Amesbury *et al.* 2018). The small 'ubiquitous' flagellate *Neobodo designis* (Skuja) Vickerman, 2004 has been shown to be a paraphyletic entity composed of organisms that have been characterized molecularly and inhabit dramatically different ecosystems (Koch and Ekelund 2005), but no species description has been undertaken as yet.

Previous morphological works had already hinted that *C. ampulla* was actually a shadow species, composed of several independent taxonomic units, rather than a single species (Todorov *et al.* 2009). The application of molecular approaches to the systematics of the family Cyphoderiidae proved that what was considered until now *C. ampulla* was formed by different polyphyletic clades (Heger *et al.* 2010, 2011). A more recent work also showed that these clades had different evolutionary histories, including transgressions of the salinity barriers that induced cladogenesis events (González-Miguéns *et al.* 2022). Here, we take the needed taxonomic actions to resolve the shadow species *C. ampulla*.

Salinity barrier transgressions structure the evolutionary history and systematics of Cyphoderiidae

The salinity barrier played a major role in the evolutionary history of Cyphoderiidae (González-Miguéns *et al.* 2022). At least four independent transitions from marine to freshwater systems have likely occurred during the Miocene, structuring the shell morphology and the ecology of the different clades (González-Miguéns *et al.* 2022). Thus, each ecological transition shapes the evolutionary history of the clades, accompanied by new biotic interactions and selective pressures. In that sense, marine clades (D and E) represent branches in Cyphoderiidae evolutionary history which developed separately from the freshwater clades during the Miocene. Moreover, morphometric analyses of the shell general shape separated efficiently all clades, except clades A and C whose resemblance could be due to convergent evolution to similar selective pressures in identical environments. Nevertheless, these clades can be easily differentiated by the disposition of their scales, imbricated (C) or not (A)—a constant character shared by all subclades (Heger *et al.* 2010, 2011, González-Miguéns *et al.* 2022). Given the profound differences in ecology, genetics, and morphology found between the clades, and its independent evolutionary history based on the ecological transitions we decided to raise groups A–E to genus status.

The first task consisted of deciding which clade would be adscribed to the genus *Cyphoderia*. Following the *C. ampulla* original description "'*D. lorica oblonga clavata, punctorum seriebus obliquis eleganter notata, hyaline, ostiolo ovato. Magn. 1/26' Dr. Werner Salisburgi detexit*" by Ehrenberg (1840), the morphology

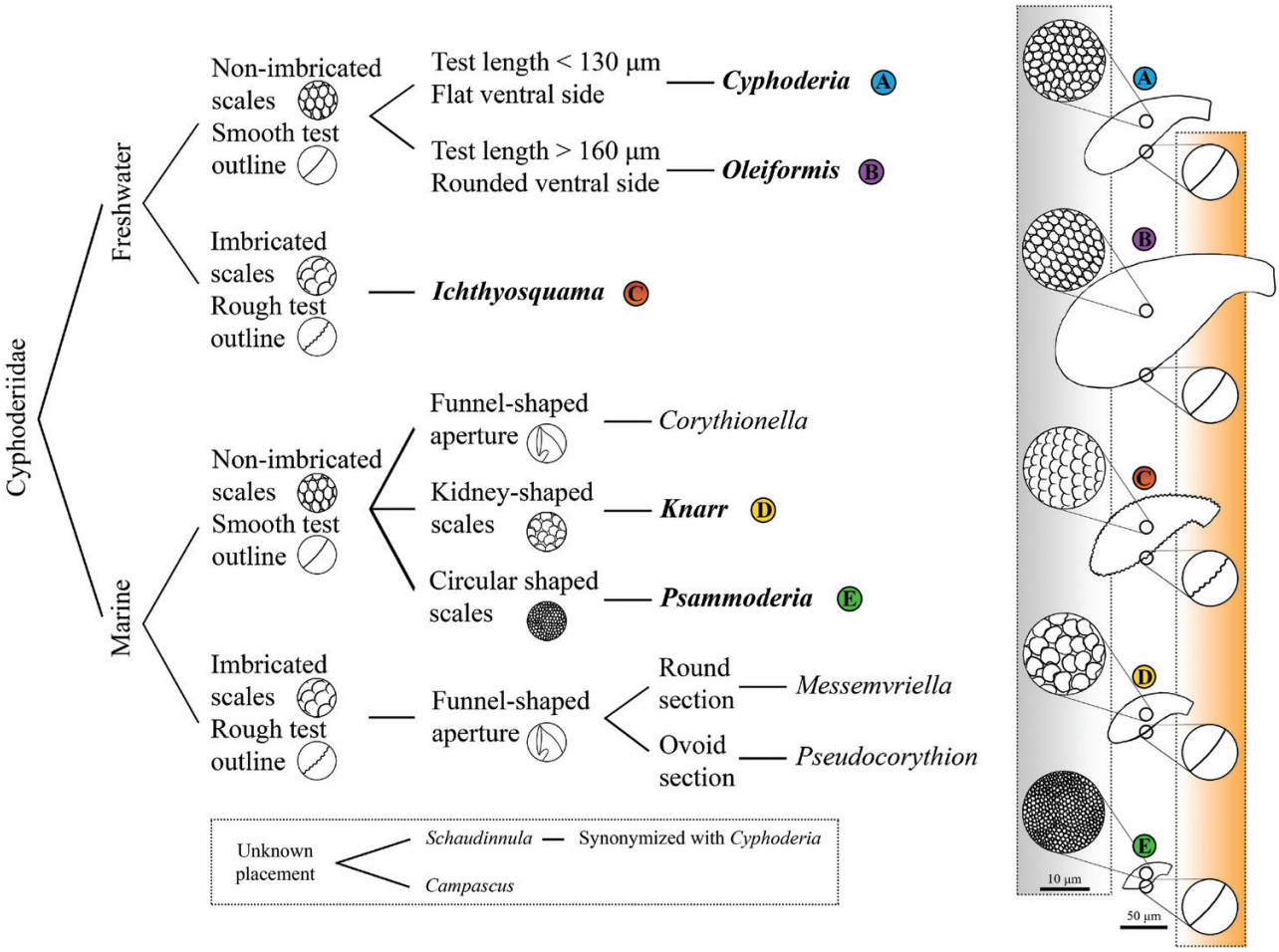


Figure 3. Dichotomous key of the family Cyphoderiidae. New genera are in bold with their respective shell outline drawing (50 µm scale bar), scale arrangement under SEM (grey box with 10 µm scale bar), and shell outline under light microscopy (orange box) in order to differentiate them. (A) ***Cyphoderia***; (B) ***Oleiformis* gen. nov.**; (C) ***Ichthyosquama* gen. nov.**: (D) ***Knarr* gen. nov.**, (E) ***Psammoderia* gen. nov.**

could fit to lineages on both clades A and C. However, in the illustration, scales were reported as non-imbricated (i.e. formally 'MB_ED_0431', on the Museum für Naturkunde Berlin portal https://portal.museumfuernaturkunde.berlin/detail/fc8b3d24797ce1dcfcf7; Fig. 4), which implies that only clade A may correspond to Ehrenberg's observations and should be named *Cyphoderia*. Therefore, the other clades (B–E) had to be newly named and characterized; the other freshwater clades were called, respectively, *Oleiformis* (B) and *Ichthyosquama* (C), and the marine clades *Knarr* (D) and *Psammoderia* (E) (Supporting Information, Appendix S1; Table S2).

Clade A, genus Cyphoderia (Figs 3, 4)

Clade A is composed of three subclades A1–A3 (Fig. 4) living in freshwater or associated with wet soil mosses or *Sphagnum*. They have a shell with non-imbricated scales and are notably smaller than Clade B. Under a light microscope a smooth shell outline is visible.

Clade B, Oleiformis gen. nov. (Figs 3, 5)

Clade B is composed of two subclades (B1 and B2) whose scales are non-imbricated and a smooth shell outline is visible under a light microscope. They inhabit freshwater or are associated with bryophytes such as *Sphagnum* or other mosses and have a typical balloon-shaped shell. The shells of the only two species known to date are typically 50–100 µm larger than the largest known species in the sister genus *Cyphoderia*.

Clade C, Ichthyosquama gen. nov. (Figs 3, 6)

Clade C is composed of five subclades (C1, C2, C3, C4, and C5) living in freshwater or associated with wet mosses or *Sphagnum*. They have a shell with imbricated scales; their sizes are comparable those of the genus *Cyphoderia*.

Clade D, Knarr gen. nov. (Figs 3, 7)

Clade D is composed of a single known subclade (D) living in marine and brackish sediments. They have a smooth shell outline visible under a light microscope with non-imbricated scales, which have a characteristic kidney shape.

Clade E, Psammoderia gen. nov. (Figs 3, 7)

Clade E is composed of a single known subclade (E) with a smooth outline shell visible under a light microscope and imbricate scales. They live in marine and brackish sediments.

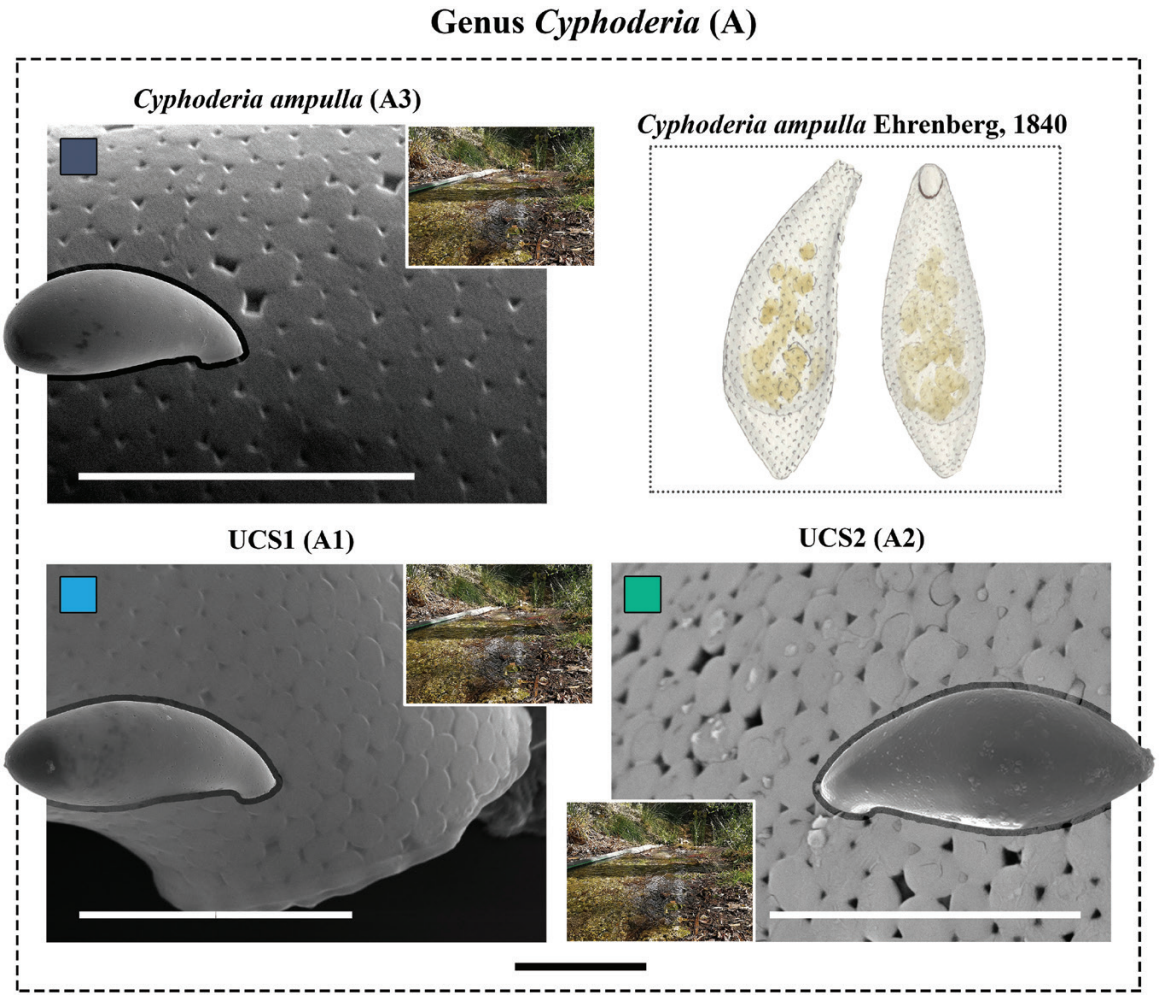


Figure 4. SEM images of *C. ampulla* and the different UCS from the *Cyphoderia* genus. Insets show the typical habitat for each organism. The original drawing of C. ampulla from Ehrenberg, 1840 is shown top right. The white bars represent a scale of 10 µm and the black one 50 µm for each organism.

Putting order into the *C. ampulla* hotchpotch

Previous studies have demonstrated the independence of lineages in *C. ampulla*, which was shown to be polyphyletic and to contain several independent taxonomic units, such as *C. trochus* (now *Ichthyosquama trochus*) or *C. major* (now *Oleiformis major*) (Heger *et al.* 2010, 2011, González-Miguéns *et al.* 2022). However, it has taken more than 10 years to describe the evolutionary history of these lineages and to develop hypotheses on their evolutionary history (González-Miguéns *et al.* 2022).

We relied on an integrative approach where genetic, morphological, and ecological data were used to describe the different taxonomic units in species [see Lara *et al.* (2020) for examples of amoeboid organisms]. The criteria used to separate the species here are based on morphometrics, when possible; otherwise, genetic distances had to be used as a criterion.

Genus Cyphoderia *Soler-Zamora, Useros, González-Miguéns & Lara (Fig. 4)*

Subclades A1, A2, and A3 share an almost identical test outline which resembles the original description of *Cyphoderia ampulla* (Ehrenberg, 1840) Leidy, 1879. All three subclades have been found together in the same location, sediment from the Morollón River in the Fuente de la Gitana recreational area (La Peza, Spain); A3 was also found in Germany, near the city of Aachen (Wylezich *et al.* 2002) and A1 was also discovered in Geneva (Switzerland). These clades are closely related (Fig. 1), with low *COI* genetic distances between them of 0.0473 (Supporting Information, Table S1). The separation between these three clades remains unclear; while they form three fully supported clades, they cannot be separated unambiguously from each other based on morphometrics because data on A2 and A3 are still insufficient (Fig. 2; Tables 1, 2). Organisms have been isolated from localities in diverging climatic zones, in both lentic and lotic systems with different limnological parameters; more data are needed to define their ecological preferences and geographical distributions. Because of these uncertainties, we consider these organisms as UCS. However, as *C. ampulla* is the type species of the genus *Cyphoderia*, one of the three subclades needs to be chosen as the type and the rest will remain UCS until more data are available to merge them or split them from *C. ampulla*. A3 has been found in a small stream near Aachen in Germany, which is the ecosystem that most resembles the site where Ehrenberg isolated the material

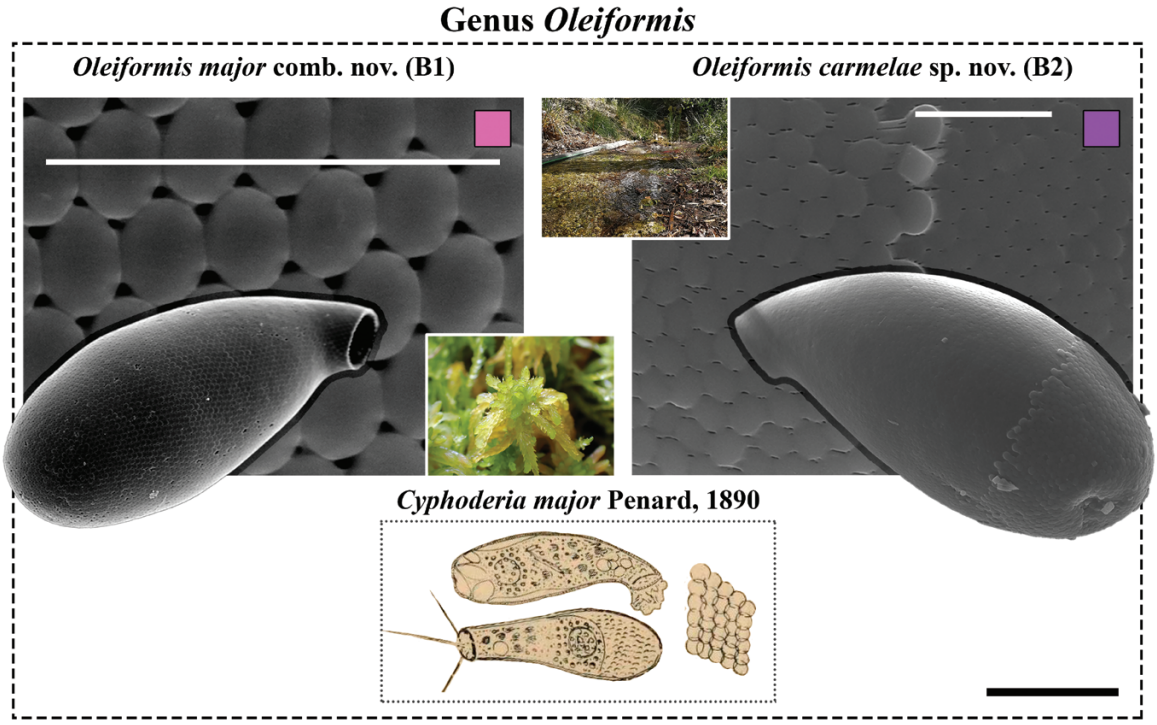


Figure 5. SEM images of the different species of ***Oleiformis* gen. nov.** Insets show the typical habitat for each organism. At the bottom, an original drawing of the previously known species is shown. The coloured squares at the top left of each image have the same colour as the clades to which each taxon corresponds in Fig. 1. The white bar represents a scale of 10 µm and the black bar 50 µm for each organism.

which allowed him to describe the species ('a swamp'); it then becomes the type species.

Oleiformis Soler-Zamora, Useros, González-Miguéns & Lara gen. nov. (Fig. 5)

Subclades B1 and B2 share similar morphology and ecology. These organisms resemble the species *Cyphoderia major* (Penard, 1890) Todorov *et al.* 2009, originally described from Wiesbaden, Germany. Subclade B1 was found in *Sphagnum* mosses and has a similar size to *C. major* (original description 175–220 µm length, 75–81 µm width; subclade B1 143.01–213 µm length, 70.37–85 µm width). However, subclade B2 is smaller (169.92–190.83 µm length, 67.44–83.14 µm width) and has larger scales (Fig. 5 B2). It was found in the sediment of the Morollón River (La Peza, Spain). Moreover, both subclades can be differentiated molecularly (Fig. 1), with a 0.0681 *COI* molecular distance. These molecular and morphological data (Fig. 2) indicate that these subclades are different taxonomic units, and therefore we describe each of these clades as *Oleiformis major* (B1) and *Oleiformis carmelae* (B2).

Ichthyosquama Soler-Zamora, Useros, González-Miguns & Lara gen. nov. (Fig. 6)

Subclade C1 (Fig. 6 C1) morphology matches with *Cyphoderia amphoralis* (Wailes & Penard, 1911) Todorov *et al.* 2009, as both test shape and dimensions fit with the original description (original description 87–153 µm; subclade C1 87.50–170 µm). In this study, we found subclade C1 in *Sphagnum* mosses (Rila, Bulgaria and Peña Yerre, Spain) and in freshwater sediments (Lake Sanabria, Spain). In the manuscript that includes its description, *C. amphoralis* was found in the marshes of Rouelbeau, Etrembiéres, Bernex and on the shores of Lake Geneva, near Pointe-à-la-Bise (Switzerland and neighbouring France). Based on these ecological and morphological data we assign this subclade to *C. amphoralis*, which becomes now *Ichthyosquama amphoralis*.

Subclade C5 (Fig. 6 C5) has been found in wet mosses from the Marchairuz Pass, Switzerland. Its morphology fits partially with the description of *Cyphoderia trochus* Penard, 1899 (original description 110–114 µm; clade C5 113.08–125.05 µm); the *terra typica* was located not far from where the organisms described here were isolated. The fundus of that species is unmistakable. On these grounds, we assign this subclade to *C. trochus*, now *Ichthyosquama trochus*.

Subclades C2, C3, and C4 (Fig. 6 C2, C3, C4) were classified as *C. ampulla* based on the overall test morphology (Todorov *et al.* 2009). However, members of these subclades have the typical imbricated scales that differentiate *Cyphoderia* from *Ichthyosquama*. Considering subclades C1 (*Ichthyosquama amphoralis*) and C5 (*Ichthyosquama trochus*) as independent species, subclades C2, C3, and C4 need to be described formally as well as based on their phylogenetic position. They are composed of sequences recovered from organisms in wet mosses and sediment from freshwater ecosystems in Spain, Bulgaria, and Canada. C2 was collected together with mosses from Vitosha (Bulgaria) and sediment from Lake Sanabria (Spain). C3 has a larger distribution area, as it includes aquatic mosses from Cape Breton (Canada), *Sphagnum* mosses from Peña Yerre (Spain), and sediment from Lake Sanabria (Spain). C4 was found in *Sphagnum* mosses from the Rhodopes

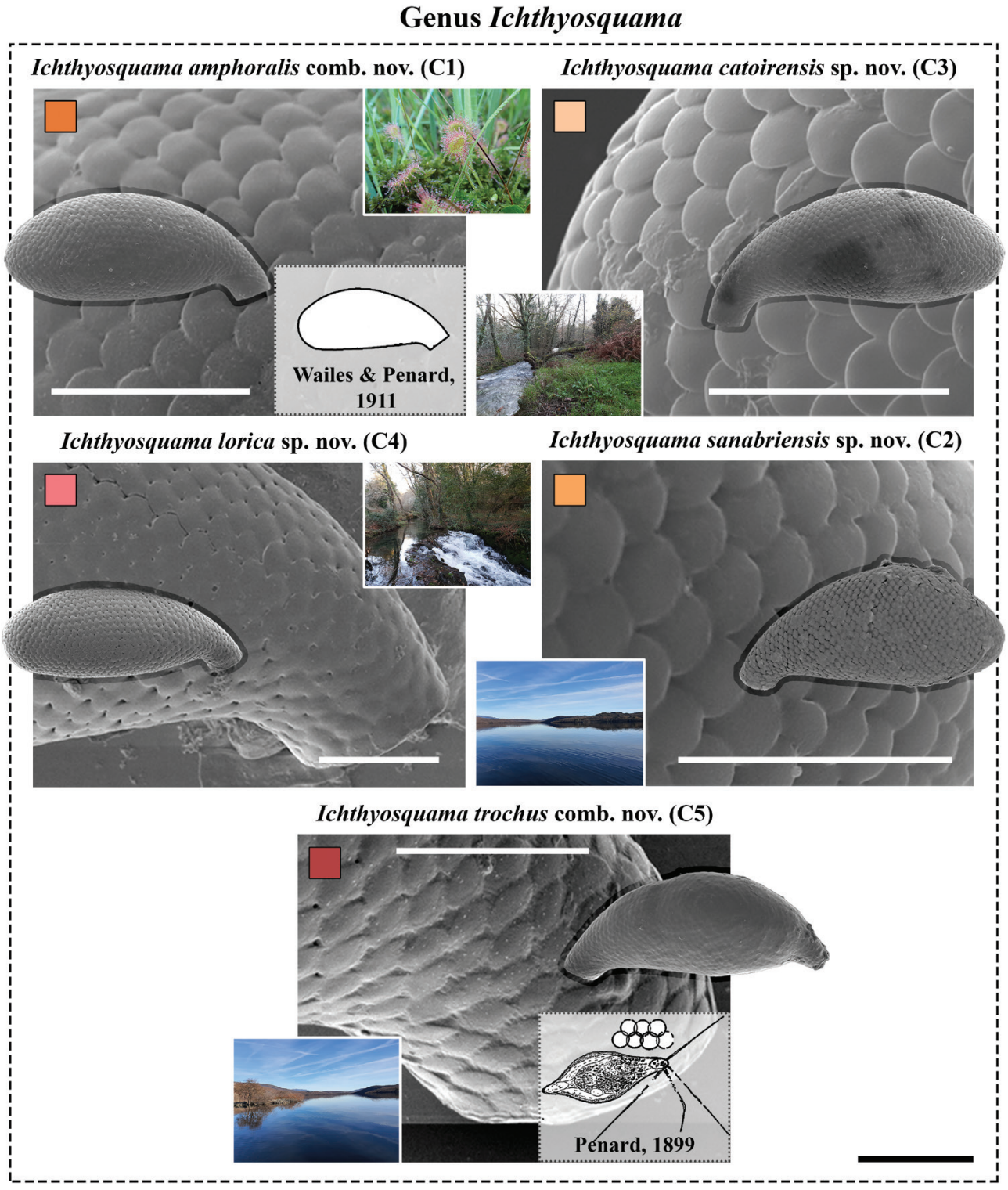


Figure 6. SEM images of the different species of ***Ichthyosquama* gen. nov.** Insets show the typical habitat for each organism. At the bottom right of the two previously known species, the original drawing is represented. The coloured squares at the top left of each image have the same colour as the clades to which each taxon corresponds in Fig. 1. The white bar represents a scale of 10 μm and the black bar 50 μm for each organism.

Mountains (Bulgaria) and river sediment collected in Catoira (Spain). Common ecological, molecular (Fig. 1; Supporting Information, Table S1), and morphometrical data (Fig. 2) separate each of these subclades, therefore, we described and named the subclades C2, C3, and C4 as *Ichthyosquama sanabriensis*, *Ichthyosquama catoirensis*, and *Ichthyosquama loricaria*, respectively.

Knarr Soler-Zamora, Useros, González-Miguéns & Lara gen. nov. (*Fig. 7*)

Subclade D (Fig. 7 D) was found in the underground waters of a marine sandy beach in Galata, Bulgaria. It fits morphologically with *Cyphoderia compressa* as described by Golemansky, 1979 from the Black Sea, Bulgaria (original description 92–112 μm; clade D, 97–111 μm). Based on the proximity of its *terra*

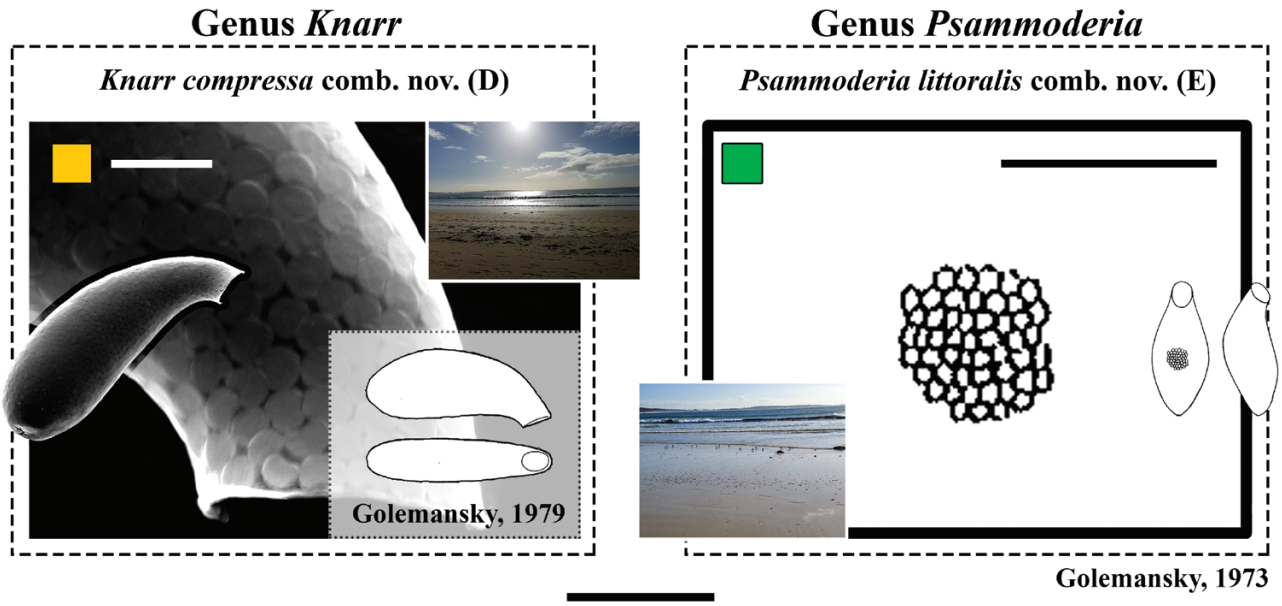


Figure 7. SEM images of the different species of ***Knarr* gen. nov.** and the original drawings of both genera, ***Knarr* gen. nov.** and ***Psammoderia* gen. nov.** Insets show the typical habitat for each organism. The coloured squares at the top left of each image have the same colour as the clades to which each taxon corresponds in Fig. 1. The white bar represents a scale of 10 µm and the black bar 50 µm for each organism.

typica from Golemansky's original recollection site and on the morphological similarity, we assign the same species name to subclade D: *Knarr compressa*.

Psammoderia Soler-Zamora, Useros, González-Miguéns & Lara gen. nov. (Fig. 7)

The morphology of subclade E (Fig. 7 E) fits perfectly with *Cyphoderia littoralis* Golemansky, 1973 as both test shape and dimensions fit with the original description (original description 38–51 µm; clade E, 46–55 µm). This marine species was collected in the underground waters of a marine sandy beach in Galata, Bulgaria, which can be considered a similar habitat to the *terra typica* of *C. littoralis* (brackish waters of sandy beaches from Miedzyzdroje, Poland). We refer to this clade as *Psammoderia littoralis*.

TAXONOMIC ACTIONS AND SPECIES ACCOUNTS

Order Euglyphida Copeland, 1956 emend Cavalier-Smith, 1997

Family Cyphoderiidae Saedeleer, 1934

Included taxa: See Supporting Information, Appendix S1.

Genus *Cyphoderia* Schlumberger, 1845

(Figs 1, 3, 4)

Type species : *Cyphoderia ampulla* (Ehrenberg, 1840) Leidy, 1879.

Updated diagnosis : Non-imbricated scales, elongated shape. Freshwater habitat.

Included taxa : See Supporting Information, Appendix S1.

Cyphoderia ampulla (Ehrenberg, 1840) Leidy, 1879

(Figs 1, 4 A3)

Type locality : Morollón River, Fuente de la Gitana recreational area, La Peza, Spain (37º 51′ N 2º 47′ W).

Habitat : River sediment.

Measurements : 88.81–123.55 µm length, 33.48–50.46 µm width, 15.54–24.50 µm pseudostome large axis, and 1.86–2.25 × 1.40–1.73 µm scales from the middle of the shell (N = 84).

Specific diagnosis : Elongated shell with a circular section and rounded bottom. Short neck at a different angle to the shell. Rounded pseudostome with a thin organic layer and teeth around it. Elliptical scales placed next to each other, more or less well aligned.

Note : Has been also isolated in the locality of Sieben Quellen, near Aachen, Germany.

Holotype : MA-Amoeba 11263.

UCS1

(Figs 1, 4 A1)

Type locality: Morollón River, Fuente de la Gitana recreational area, La Peza, Spain (37º 51′ N 2º 47′ W).

Habitat : Lake and river sediment.

Measurements : 96.32–127.20 µm length, 38.24–44.24 µm width, 14.96–17.46 µm pseudostome large axis, and 1.88–2.01 × 1.41–1.43 µm scales from the middle of the shell (N = 5).

Specific diagnosis : Elongated shell with a circular section and rounded bottom. Short neck at a different angle to the shell. Rounded pseudostome with a thin organic layer and protruding scales around it. Elliptical scales placed next to each other, in no particular order; scales are larger in the middle of the shell than at the extremes.

Note : Has been also isolated in Lake Geneva at the locality of Préverenges, Canton Vaud, Switzerland.

Holotype : MA-Amoeba 11261.

UCS 2

(Figs 1, 4 A2)

Type locality : Morollón River, Fuente de la Gitana recreational area, La Peza, Spain (37º 51′ N 2º 47′ W).

Habitat : River sediment.

Measurements : 88.24–138.73 µm length, 40.13–54.29 µm width, 14.33–21.12 µm pseudostome large axis, and 2.14 × 1.45 µm scales from the middle of the shell (N = 7).

Specific diagnosis : Elongated shell with a circular section and slightly rounded fundus. Short neck at the same angle as the shell. Rounded pseudostome with a thin organic layer surrounding it. Elliptical scales placed next to each other, in no particular order, but scales in the middle of the shell tend to be larger than at the extremes.

Holotype : MA-Amoeba 11262.

Ichthyosquama Soler-Zamora, Useros, González-Miguéns & Lara gen. nov.

(Figs 1, 3, 6)

Zoobank registration: urn:lsid:zoobank.org:act:477C449F-3996-428E-932C-16B1EEC4BDFA.

Type species : *Ichthyosquama amphoralis* (Penard, 1905) comb. nov.

Description : Scales from the shell are imbricated and the species inhabit in freshwater ecosystems.

Etymology : A contraction of ἰχθύς ichthys (fish in Ancient Greek) and squama (scale in Latin). The genus name describes the imbricated scales of members of this genus, which are reminiscent of those of a fish.

Included taxa : See Supporting Information, Appendix S1.

Ichthyosquama amphoralis (Penard, 1905) comb. nov.

(Figs 1, 6 C1)

Zoobank registration: urn:lsid:zoobank.org:act:CA3A8AC0-5410-43B4-B80D-E4EC47E7D75B.

Ichthyosquama catoirensis Soler-Zamora, Useros, González-Miguéns, Blanco-Rotea, Carrasco-Braganza, Salvador-Velasco, Guillén-Oterino, Tenorio-Rodríguez & Lara sp. nov.

(Figs 1, 6 C3; Model 1)

Zoobank registration: urn:lsid:zoobank.org:act:8996A03C-3944-4FF2-A53E-B8133191F313.

Type locality : Catoira, Galicia, Spain (42º 40′ N 8º 43′ W).

Habitat : Freshwater sediment and mosses.

Measurements : 116.21–146.77 µm length, 45.80–71.80 µm width, 16.17–19.62 µm pseudostome large axis (N = 9).

Specific diagnosis : Elongated test composed of imbricated scales, bent neck.

Intraspecific variability : Large variability in the general shape of the test. Most have the typical shape of the genus, with an oval, slender test, a flatter ventral side, a rounded fundus, and a curved neck. However, the shape can be more rhomboidal and the fundus can be pointier or flatter.

Differential diagnosis : *Ichthyosquama catoirensis* can be diagnosed from other members of the genus by its specific sequence of the mtDNA marker and its phylogenetic position. The morphometric analyses performed in this work can separate this species from other members of the genus.

Etymology : We propose this name as a reference to the type locality Catoira (Galicia, Spain).

Holotype : MA-Amoeba 11265.

Ichthyosquama loricaria Soler-Zamora, Useros, González-Miguéns & Lara sp. nov.

(Figs 1, 6 C4)

Zoobank registration: urn:lsid:zoobank.org:act:496DE5E9-54CD-43F4-82EB-74BB3CB508C4.

Type locality : Rhodopes, Bulgaria (41º 59′ N 24º 10′ E).

Habitat : Freshwater sediment and mosses.

Measurements : 96.25–129 µm length, 36.30–47 µm width, 13.18–16.38 µm pseudostome large axis (N = 108).

Specific diagnosis : Elongated test composed of diagonal rows of round, imbricated scales. The scales are generally partially fused together. Rounded fundus. The ventral side is slightly flattened. There is a thin layer of cement around the aperture and in two individuals we could count a series of 16–17 tear-shaped scales around the interior side of the aperture. The size of the visible part of the scales is generally 3.12–3.77 × 2.62–3.11 µm.

Differential diagnosis : *Ichthyosquama loricaria* can be diagnosed by its specific sequence of the mtDNA marker and its phylogenetic position. The presence of tear-shaped scales around the inner part of the aperture can differentiate it from *I. amphoralis*. We did not observe the presence of this trait in other species.

Etymology : The epithet refers to the catfish family Loricariidae and to the genus *Loricaria* based on its resemblance to these animals due to the imbricated scales. The lateral position of the aperture is reminiscent of the sucker mouth typical of members of the Loricariidae. Loricaria also means coat of mail in Latin, which could refer to the test of imbricated scales.

Holotype : BG-IBER-INV-000016458.

Ichthyosquama sanabriensis **Soler-Zamora, Useros, González-Miguéns, Blanco-Rotea, Carrasco-Braganza, Salvador-Velasco, Guillén-Oterino, Tenorio-Rodríguez & Lara sp. nov.**

(Figs 1, 6 C2)

Zoobank registration: urn:lsid:zoobank.org:act:49306CC6-C109-4647-946F-221358100DB7.

Type locality : Lake Sanabria, Spain (42º 10′ N 6º 44′ W).

Habitat : Freshwater sediment and mosses.

Measurements : 102.93–150.35 µm length, 38.82–60.03 µm width, and 14.30–19.89 µm pseudostome large axis. The size of the visible part of the scales is generally 3.22–3.91 × 2.47–2.92 µm (N = 109).

Specific diagnosis : Elongated test composed of diagonal rows of imbricated scales. Posterior end generally rounded and ventral side generally flattened. In two specimens, we counted 17 oral scales.

Intraspecific variability : We found some individuals in which the outline of the test was somewhat rhomboidal, or slightly flattened at the very end. One particular (abnormal?) specimen had its test bent like a sickle, thus having a concave ventral side.

Differential diagnosis : *Ichthyosquama sanabriensis* can be diagnosed by its specific sequence of the mtDNA marker and its phylogenetic position. The morphometric analyses performed in this work separates this species from other members of the genus.

Etymology : We propose this name as a reference to the type locality, Lake Sanabria (Castilla y León, Spain).

Holotype : MA-Amoeba 11266.

Ichthyosquama trochus **(Penard, 1899) comb. nov.**

(Figs 1, 6 C5)

Zoobank registration: urn:lsid:zoobank.org:act:427AF3BE-7265-4771-A7F6-C2318FED2536.

Knarr **Soler-Zamora, Useros, González-Miguéns & Lara gen. nov.**

(Figs 1, 3, 7 D)

Zoobank registration: urn:lsid:zoobank.org:act:A5C3849A-0CE6-4411-9E03-C8C5A22E7C0E.

Type species : *Knarr compressa* (Golemansky, 1979) comb. nov.

Description : Scales from the shell are non-imbricated with a kidney shape and the species inhabits marine ecosystems.

Etymology : A knarr was a type of Norse merchant ship used in long sea voyages. The name refers to the shape of the shells, and also to the wide geographical distribution of the genus (González-Miguéns *et al.* 2022).

Included taxa : See Supporting Information, Appendix S1.

Knarr compressa **(Golemansky, 1979) comb. nov.**

(Figs 1, 7 D)

Zoobank registration: urn:lsid:zoobank.org:act:9325FB59-68C8-43B2-A556-401D4F4FEE0F.

Oleiformis **Soler-Zamora, Useros, González-Miguéns & Lara gen. nov.**

(Figs 1, 3, 5)

Zoobank registration: urn:lsid:zoobank.org:act:D473BEDF-9357-4AD7-81DA-123888857181.

Type species : *Oleiformis major* (Penard, 1890) comb. nov.

Description : Scales from the shell are non-imbricated, the species inhabits in freshwater ecosystems and they have a balloon-shaped shell that measures between 50–100 µm more than the largest species in the sister genus *Cyphoderia*.

Etymology : The genus name refers to the shape like an olive.

Included taxa : See Supporting Information, Appendix S1.

Oleiformis carmelae **Soler-Zamora, Useros, González-Miguéns & Lara sp. nov.**

(Figs 1, 5 B2; Model 2; Supporting Information, Fig. S1)

Zoobank registration: urn:lsid:zoobank.org:act:CD3A3A3F-B2FC-4FBF-9B79-6F5EDC99CB6C.

Type locality : Morollón River, Fuente de la Gitana recreational area, La Peza, Spain (37º 51′ N 2º 47′ W).

Habitat : River sediment.

Measurements : 169.92–190.83 µm length, 67.44–83.14 µm width, 22.51–27.85 µm pseudostome large axis, and

2.65–2.99 × 1.93–2.26 μm scales from the middle of the shell (N = 13).

Specific diagnosis : Elongated shell with an almost perfectly circular section. The shell is larger towards its middle part, giving an inflated appearance; rounded fundus. Short neck in comparison to other members of Cyphoderiidae. Rounded pseudostome with a thin organic layer surrounding it. Elliptical scales placed next to each other in a structured fashion; each scale is surrounded by six pores.

Intraspecific variability : Some individuals have a slightly more pointed fundus (Supporting Information, Fig. S1).

Differential diagnosis : It can be confused with *O. major* because of their similar shell shape. *O. carmelae* is smaller, with relatively larger scales. It differs also genetically in both *COI* and *SSU*.

Etymology : In honour of Professor Carmen Zamora-Muñoz, mother of the first author.

Holotype : MA-Amoeba 11264.

Oleiformis major (Penard, 1890) **comb. nov.**

(**Figs 1**, **5** B1)

Zoobank registration: urn:lsid:zoobank.org:act:AE0C90E5-D1D6-431D-826C-511EB2B162E3.

Psammoderia **Soler-Zamora, Useros, González-Miguéns & Lara gen. nov.**

(**Figs 1**, **3**, **7** E)

Zoobank registration: urn:lsid:zoobank.org:act:62900003-409E-4E2C-B018-6AAAC15131F1.

Type species : *Psammoderia littoralis* (Golemansky, 1973) comb. nov.

Description : Imbricated scales; lives in the supralittoral zone of marine sandy beaches.

Etymology : The name is contraction between the Ancient Greek ψάμμος psammo meaning sand, and *Cyphoderia*.

Included taxa : See Supporting Information, Appendix S1.

Psammoderia littoralis (Golemansky, 1973) **comb. nov.**

(**Figs 1**, 7 E)

Zoobank registration: urn:lsid:zoobank.org:act:76107793-0720-4671-9BE6-BC4AED94E792.

CONCLUSION

In this manuscript, we describe the problem of shadow species, which consists in the taxonomically unfinished state of organisms where all data needed to describe the new taxa giving it a specific Linnean name are present, but no action has been taken. Thus, shadow species, species complexes, and UCS coexist in the literature making systematics unclear. This issue is especially important for non-specialists who do not follow the development of knowledge in the group. This leads to misinterpretations in taxa biodiversity, ecology, evolution, or conservation status. Moreover, the resulting inaccuracies in taxonomy have implications in the reproducibility of experiments conducted on the organisms concerned. It is therefore crucial to resolve cases of shadow species as soon as possible or, even better, to prevent their existence by describing species when data are sufficient to do so. Here, we took action based on the results of Todorov *et al.* (2009), Heger *et al.* (2010, 2011), and González-Miguéns *et al.* (2022) resolving the case of *C. ampulla* shadow species in an integrative way. We encourage such actions to take place for other eukaryotic organisms, regardless of their size.

SUPPLEMENTARY DATA

Supplementary data is available at Zoological Journal of the Linnean Society online.

Appendix S1. Synonymic list of *Cyphoderia*, ***Ichthyosquama*** **gen. nov.**, ***Knarr*** **gen. nov.**, ***Oleiformis*** **gen. nov.**, and ***Psammoderia*** **gen. nov.**

Appendix S2. 3D model design.

Figure S1. Illustration of ***Oleiformis carmelae*** **sp. nov.** intraspecific variability.

Table S1. *COI* and *SSU* genetic distance between clades A (A1, A2, A3), B (B1, B2), C (C1, C2, C3, C4, C5), D, E, and F measuring the number of base substitutions per site from averaging over all *COI* and *SSU* sequence pairs between each clade described above using the Tamura 3-parameter substitution model in the software MEGA v.10.1.8 (Kumar *et al.* 2018).

Table S2. Correction of the list of GenBank accession files used for phylogenetic analyses from González-Miguéns *et al.* (2022).

SHARED DATA

The dataset used in this paper of Gonzalez-Miguens *et al.* (2022) is in Project 4082: *Cyphoderia ampulla* available at MorphoBank: http://dx.doi.org/10.7934/P4082.

AUTHOR CONTRIBUTIONS

Carmen Soler-Zamora, Rubén González-Miguéns, and Enrique Lara designed the study; Pablo Gómez-Rodríguez built the 3D models; Carmen Soler-Zamora and Fernando Useros prepared the synonymic list of the genera *Cyphoderia*, *Ichthyosquama*, *Knarr*, *Oleiformis*, and *Psammoderia*; Carmen Soler-Zamora prepared the images and performed the analyses; Carmen Soler-Zamora interpreted and discussed the data together with Rubén González-Miguéns, Fernando Useros, and Enrique Lara; Carmen Soler-Zamora, Rubén González-Miguéns, Fernando Useros, and Enrique Lara wrote the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

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