INTRODUCTION

Bryozoans are a phylum of colonial suspension feeders composed of iterated modules termed zooids. Zooids are traditionally divided into a cystid and polypide with the former represented by the body wall and cuticle and the latter the remaining soft tissues, which can be retracted into the protective cystid (Mukai et al. 1997; Ryland, 1970; Schwaha, 2020a, 2020b; Schwaha et al. 2020). Over 6000 recent and 15,000 fossil species are currently described (Bock & Gordon, 2013) and can be systematically divided into Phylactolaemata and Myolaemata (Schwaha et al. 2020). With over 5000 described species, gymnolaemates belonging to the Myolaemata are the largest clade. Ctenostome bryozoans are a comparatively small clade of gymnolaemate bryozoans with merely 350 species described, whereas the large bulk belongs to the Cheilostomata (Taylor & Waeschenbach, 2015). Ctenostomes are paraphyletic with cheilostomes originating from a ctenostome-like ancestor (Taylor & Waeschenbach, 2015; Todd, 2000; Waeschenbach et al. 2012). There are only few, gymnolaemate-specific characters such as parietal muscles, specific interzooidal...
pore complexes and a collar that characterize this clade (Schwaha et al. 2020, Schwaha, 2020b). Traditional systematics recognize around seven main superfamilies of ctenostomes that are primarily distinguished by characters of zooidal and colony shape (Jebram, 1973, 1986; Todd, 2000), subordinately on specific details of their internal morphology. The recent advent of more thorough soft-body morphological studies, however, indicates that such characters harbor a wealth of systematically and taxonomically important details (see Schwaha et al. 2020), previously little used for ctenostome taxonomy (e.g., Braem, 1939, 1940, 1951; Jebram, 1973, 1986; Porter & Hayward, 2004).

The superfamiliy Alcyonidioidae represents one of the largest taxa of ctenostomes, mostly owing to the species-rich and diverse genus Alcyonidium that currently houses over 80 described species, with numbers rising (www.bryozoanet/alcyoniidiae/alcyonidium.html). Although the inclusion of some clades has not been clarified, at least five families can be distinguished: Alcyonidiidae, Clavoporididae, Flustrellididae, Pachyrozoa and Pherusellidae. Clavoporids and pachyzooids are deep-sea forms with only a few species currently described, whereas the other three families form encrusting to erect colonies consisting of tightly arranged zooids with usually little to moderate peristome sizes (Schwaha, 2020a). Flustrellididae and Pherusellidae are little investigated, but share a rectangular to bilateral apertural area and, as far as investigated, a unique pseudocyphonautes larva (Decker et al. 2020; Reed, 1991). Additional information is extremely scarce, except for Flustrellidra hispida, which, owing to its easy access and abundance in the intertidal area, has been subject to many investigations (e.g., Best & Thorpe, 1983, 1996; d’Hondt, 1977; Kvach et al. 2019; Markham & Ryland, 1991; Waeschenbach et al. 2006).

Pherusellids are a small family of currently three described species in one genus Pherusella tubulosa (Ellis & Solander, 1786), P. flabellaris (Kirkpatrick, 1890) and P. brevituba Soule, 1951 from various locations. A recent study on the detailed morphology and life cycle of an interesting epiphytic species of Pherusella revealed that it represents a new species awaiting its formal description (Decker et al. 2020). Detailed studies on pherusellids are few and old (Prouho, 1892; Soule, 1951, 1953) and the significant lack of any recent detailed studies not only called for a formal description, but a complete revision of the entire family. Consequently, the current study comprises the revision of the family by analyzing three additional species of Pherusella, including the holotype of P. flabellaris and a second new species.

2 | MATERIAL AND METHODS

2.1 | Material

Specimens of Pherusella liowae sp. nov. were collected in 2019 at Changi Beach and Pulau Ubin (Ubin Island), Singapore, on various substrata. Samples were fixed and stored in 96% ethanol and sent to the University of Vienna for further investigations. Voucher specimens are deposited at Lee Kong Chian Natural History Museum, National University of Singapore, accession numbers ZRC.BRY.0897 (holotype), ZRC.BRY.0898 (paratype) (ZRC=Zoological Reference Collection). An additional paratype is deposited at the National Institute of Water and Atmospheric Research (NIWA), accession number: NIWA 146102.

Specimens of Pherusella minima sp. nov. were collected during 2018 and 2019 in Pula, Croatia. Specimens for histological investigations were fixed in 2.5% glutaraldehyde (GA) in 0.1 M phosphate buffer (PB) (pH = 7.4). Subsequently, samples were rinsed at least three times in 0.1 M PB for 15 min each and stored at 4°C in PB with additional 0.1% NaN3. Supplementary paratype material and specimens for molecular investigations were fixed and stored in 96% ethanol. Type specimens are deposited at the Bavarian State Collection of Zoology (Zoologische Staatssammlung München (ZSM)), accession numbers ZSM-20200454, ZSM-20200455, ZSM-20200456, ZSM-20200457. Additional analyzed specimens of P. minima sp. nov. were taken from the Natural History Museum of London (NHMUK 1975.1.12.331).

The investigated specimens of Pherusella tubulosa (Ellis & Solander, 1786) (1966.12.29), Pherusella flabellaris (Kirkpatrick, 1890) (1889.8.21.128) and Pherusella brevituba Soule, 1951 (1963.9.4.1) are all part of the Bryoza collection of the Natural History Museum in London (NHMUK). Small pieces comprising several zooids each were cut from the colonies and transferred to Vienna for photographic documentation and histological processing.

2.2 | Morphological analyses

Stereomicroscopic images were either taken with a Nikon J1 camera (Nikon, Tokyo, Japan) mounted on a Wild M420 stereomicroscope (Wild Heerbrugg, Heerbrugg, Switzerland) or with a DsRi2 microscope camera mounted on a Nikon SMZ25 stereomicroscope. Some specimens fixed in 2.5% GA were embedded into Agar Low Viscosity Resin (LVR, Agar Scientific Ltd., Stansted, UK). Sectioning into 1 μm semithin section series was carried out using a Leica UC6 ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany) and a Histo-Jumbo diamond knife (Diatome AG, Biel, Switzerland). Ribbons of semithin sections were stained with 0.1% toluidine blue for 30 s at 60°C. Afterward, images of semithin section series were either obtained with a Nikon NiU compound microscope equipped with a DsRi2, a Nikon E600 compound microscope with an Olympus DP27 camera, or a Leitz Orthoplan with a Nikon J1 camera. Before the image series were imported into the 3D-reconstruction software Amira 6.3 (FEI, Oregon, USA), they were converted into grayscale.

The AlignSlices tool of Amira was used to align the image series prior to semi-manual segmentation. Segmented structures were reconstructed as surface models and surrounding structures as volume rendering before images were taken with the snapshot function in Amira.

2.3 | Measurement of zooid dimensions

Zooid dimensions were measured with the microscope-supplied NIS Elements software (Nikon, Tokyo, Japan). Zooid length and width were
measured from the frontal side, zooid length along the longitudinal axis and zooid width along the widest part of the zooid. Peristome length was measured from the lateral side from the edge of the orifice to the transition area between peristome and frontal wall.

2.4 | Additional Software

Distribution map was generated with GeoMapApp (www.geomapapp.org)/CC BY.
Family Pherusellidae Soule in Osburn, 1953

**Amended diagnosis.** Colonies either encrusting, or erect bilaminar from encrusting base. Cystid cuticular, semi-transparent to yellowish, brownish in color (Figures 1a, b; 2a, b; 3a, b; 4a, b; 5a, b; 6a, b), often overgrown. Cuticle thick and multilayered (Figure 7). Vertical wall commonly folded (Figure 8b, c). Zooids oval in young colonies, irregularly rectangular to polygonal in older colonies (Figure 4b, c). Zooids arranged in alternating rows (Figures 5b, 6b). Zooid size ranges from 800 µm to 1300 µm in length and 230 µm to 600 µm in width (Figure 9). Orifice/apertural area square to quadrangular with four prominent folds, on tubular peristome. Peristome short to long (Figure 1b; 2b; 3b; 4b; 8a-e). Polymorphic zooids absent. Ancestrula with remains of larval valves on frontal wall throughout ontogeny (Figure 10). Collar distal of diaphragmatic sphincter, short, membranous. Diaphragm always basal with multiple proximal folds in retracted zooids (Figure 11). Apertural muscles a single pair of parieto-diaphragmatic muscles. Four duplicature bands, frontal bands broad, sometimes connected. Parietal muscles from basal to frontal side on each lateral side, extending from proximal to distal side (Figure 1c, d; 2c, d; 3c, d; 5c, d; 12). Lophophore bilateral with oral rejection tract, with 19–33 tentacles (Figure 9). Cardia elongated, cardiac constrictor not present. Cecum small to medium

**Figure 2.** *Pherusella flabellaris* (Kirkpatrick, 1890). Holotype (NHMUK 1889.8.21.128). (a-b) Stereomicroscopic images showing the long peristome. Three zooids containing developing embryos within their tentacle sheath. (c-d) 3D reconstruction of a zooid based on semithin sections. The body wall is visualized as volume rendering, internal structures as surfaces in different colors. Lateral-frontal view (c) and basal view (d). Abbreviations: ca—cardia, cae—cecum, cg—cerebral ganglion, db—duplicature band, e—embryo, es—esophagus, int—intestine, l—lophophore, o—orifice, pc—pore complex, pd—parieto-diaphragmatic muscle, ph—pharynx, ps—peristome, py—pylorus
in size, anus generally vestibular, fimbriate with multiple foldings (Figures 11e; 12). When present, funicular muscle from cardiac or cecal area to basal body wall (Figure 3c, d). Prominent pore complexes with chitinous reinforcing rim. Septula usually multiporous with up to three to four perforations (Figure 13). Development of three to five embryos within tentacle sheath (Figure 2b; 5b; 14d). Pseudocyphonautes larvae. Pherusellidae comprises one genus, Pherusella Soule, 1951.

Remarks. Previous systematic treatments of pherusellids included merely few characters concerning the cystid particularly zooidal dimensions and peristome length. An estimation of tentacle number range had also been previously provided (see d’Hondt, 1983). The amended diagnosis includes multiple characters from soft-tissue morphology including muscle systems, which previously have shown differences among ctenostomes (Schwaha & Wanninger, 2018) and details of the digestive tract.

3.2 Genus Pherusella Soule, 1951

Type species. Pherusa tubulosa Ellis & Solander, 1786

Diagnosis. Same as for family

Remarks. The original generic name, Pherusa Lamouroux, 1816, is preoccupied by Pherusa Oken, 1807 and was renamed Pherusella

FIGURE 3 Pherusella liowae sp. nov. (a-b) Stereomicroscopic images of a colony showing the peristome. (c-d) 3D reconstruction of a zooid based on semithin sections. The body wall is visualized as volume rendering, internal structures as surfaces in different colors. Lateral view (c) and basal view (d). Abbreviations: ca—cardia, cae—cecum, db—duplicature band, es—esophagus, fm—funicular muscle, int—intestine, l—lophophore, o—orifice, pc—pore complex, pd—parieto-diaphragmatic muscle, ph—pharynx, ps—peristome, py—pylorus
Soule, 1951. The genus *Pherusella* was previously listed among Flustrellidridae (Osburn, 1953), but can be easily distinguished by its quadrangular aperture and lack of polymorphic zooids (d’Hondt, 1983).

### 3.3 | *Pherusella tubulosa* (Ellis & Solander, 1786)

*Flustra tubulosa* Ellis & Solander, 1786 (p.17, n. 11).

*Pherusa tubulosa* (Ellis & Solander): Lamouroux, 1816 (p. 117-119, pl. 2, figure 1, A, B, C).

*Alcyonidium flustrelloides* Barroso, 1920 (p. 353-354, figure 1).

*Pherusella tubulosa* (Ellis & Solander): Soule, 1951 (p. 368).

**Type material.** Collected by Mr. Greg in the Commonwealth of Dominica, encrusting a fucoid-like alga (Ellis & Solander, 1786) (Figure 15). Ellis’s material including the holotype was located at the Royal College of Surgeons and destroyed during World War II.

**Additional material.** NHMUK (Natural History Museum London) 1966.1.2.29; limestone cliff scraping from a depth of 24.4 m, Bassassa, Malta, July 1965.

**Description.** Young colonies unilaminar, encrusting; older colonies with erect bilamellar frond-like extensions, several cm in height. Semi-transparent to yellowish, brownish in color (Figure 1a, b, often covered by epizoic organisms. Zooids longer than wide, irregularly rectangular to hexagonal, 1254 µm in length and 515 µm in width.

Long tubular erect peristome up to 824 µm in height (Figure 9). Vestibular wall weakly folded with few wrinkles. Collar and apertural muscles as for genus (Figures 1c, d; 8a; 11a). Lophophore with 30–33 tentacles (Figure 9). Particularly long cardia, distinct large cecal pouch, anus vestibular (Figures 1a, b; 12b). Multiporous pore complexes present (Figures 1c, d; 13a, b). Prominent chitinous rim surrounding septula with three–four perforations, each 1 µm in diameter (Figure 13a, b).

**Distribution.** *Pherusella tubulosa* displays a broad distribution in tropical and subtropical waters of the Atlantic Ocean as well as in the Mediterranean Sea. The type material was collected in
the Caribbean Sea at Dominica and together with later reports from Santo Domingo (Dominican Republic) (Ellis & Solander, 1786; Lamouroux, 1816). There are reported findings from the Azores (Prenant & Bobin, 1956), the whole Iberian Peninsula (Reverter-Gil et al. 2016), and throughout the Mediterranean Sea (d’Hondt, 1983; Prenant & Bobin, 1956; Prouho, 1892; Rosso & Di Martino, 2016; Rosso et al. 2019). (Figure 15). There are several additional reported findings of *P. tubulosa* from the Indian Ocean like Visakhapatnam, India (Rao & Ganapati, 1980), but species affiliation seems uncertain and probably are pherusellid species known from the South China Sea. It appears that superficially similar species with long peristomes were wrongly identified as *P. tubulosa*, implying a broader distribution range (see, e.g., d’Hondt, 1983). Furthermore, material of presumably *P. tubulosa* collected at Algeciras (Spain) was wrongly identified by Barroso (1920) as a new species, named *Alcyonidium flustrelloides*, which is therefore a junior synonym (see Reverter-Gil et al. 2016). Finally, the citation of the species from Norfolk by Woodward (1833 as *Flustra tubulosa*) really corresponds to *Jellyella tuberculata* (Bosc, 1802) (Vieira, pers. communication).
Remarks. Ellis and Solander (1786) introduced the name tubulosa for this species referring to the very long and tubular peristome. This is also reflected in the earliest English common name for the species, pipy sea matt. Accounts for the tentacle number show a high variation that range from 25–35 in general (d’Hondt, 1983), 25–30 from Mediterranean France (Prenant & Bobin, 1956), 24–26 in Iberian material (Reverter-Gil et al. 2016) and 30–33 in our own material from Malta. This calls for a wider analysis of _P. tubulosa_ from various localities in order to assess plasticity of certain morphological characters and evaluate whether multiple species might in fact be present.

### 3.4 Pherusella flabellaris (Kirkpatrick, 1890)

*Pherusella flabellaris* Kirkpatrick, 1890 (p. 23, pl. 4. figures 3, 3a).

*Pherusella flabellaris* (Kirkpatrick): Soule, 1951 (p. 368).

**Type material.** Holotype: NHMUK 1889.8.21.128; stored in Natural History Museum London; collected from Tizard reef, South China Sea, 58.5 m, 1888, collected P.W. Bassett-Smith, presented Lords of the Admiralty (Figure 15); colony was encrusting a sponge of the genus *Axinella* (Kirkpatrick, 1890).

**Description.** Colonies unilaminar, encrusting. Semi-transparent to yellowish, brownish in color (Figure 2a, b). Zooids longer than wide, irregularly rectangular to hexagonal, 1254 µm in length and 515 µm in width. Long tubular erect peristome 762 µm in height (Figure 9). Vestibular wall smooth (Figure 8b). Collar and apertural muscles as for the genus (Figure 2c, d; 11b). Lophophore with 28 tentacles (Figures 9, 14c). Long cardia, distinct cecal pouch, anus vestibular (Figures 2c, d; 12c). Entire zooidal wall multiporous with pores of about 3 µm in diameter. Prominent chitinous rim surrounding single pore (Figure 13c, d). Embryos incubated in modified, enlarged tentacle sheath, up to three embryos, serially arranged in single zooid (Figures 2b; 14d).

**Distribution.** The type material from the South China Sea remains the only reported finding of this species (Kirkpatrick, 1890) (Figure 15).

**Remarks.** The species name derivates from the Latin, *flabellaris*, fan-shaped, alluding to the colony shape with flat, soft and flexible expansion (Kirkpatrick, 1890).

### 3.5 Pherusella liowae sp. nov.

LSID: http://zoobank.org/urn:lsid:zoobank.org:act:4EDE5AA8-C81E-4A3F-AB94-2AC85C86BE17

**Type material.** Holotype: ZRC.BRY.0897 (Zoological Reference Collection), Lee Kong Chian Natural History Museum, National University of Singapore, collected from rope at Pulau Ubin, Singapore, 1.40285° N, 103.97300° E, 0 m, 10 May 2019, by Dr Lee Hsiang Liow (Figures 6a, 14). Paratype: ZRC.BRY.0898, Lee Kong Chian Natural History Museum, National University of Singapore, collected from rock at Changi Beach east, Singapore, 1.39068° N, 103.99383° E, 0 m, 9 May 2019, by D. P. Gordon. Paratype: NIWA (National Institute of Water and Atmospheric Research) 146102, same data as for holotype (Figure 15).

**Description.** Colonies unilaminar or bilaminar, encrusting with erect lobes, up to 6 cm in maximum dimensions (Figure 6a). Semi-transparent to yellowish at margins, to dark brown in older parts of colony (Figure 3a, b). Zooids very narrow, much longer than wide, irregularly rectangular to hexagonal, 887 µm in length and 233 µm in width. Peristome tubular and erect, medium size of 340 µm (Figure 9). Vestibular wall and parts of peristome associated body wall strongly fringed and wrinkled (Figures 7g, h, 8e). Collar as for the genus (Figure 11c). Duplicature bands as for the genus with frontal ones broad and interconnected (Figure 3c, d). Apertural muscles as for the genus (Figures 3c, d; 12d). Lophophore with 24–26 tentacles (Figure 9). Long cardia, small inconspicuous cecal pouch, anus vestibular (Figures 3c, d; 12d). Multiporous pore complexes with chitinous rim surrounding septum with three–four perforations, each 1 µm in diameter (Figures 3c, d; 13i, j).
Etymology. The species name honors paleoecologist and bryozoan researcher Lee Hsiang Liow, University of Oslo, who enabled collection of the material.

Distribution. The type localities on Johor Strait in the South China Sea remain the only reported sources of this species (Figure 15).

3.6 | Pherusella brevituba Soule, 1951

_Pherusella brevituba_ Soule, 1951 (p. 369, Figures 1-4).

**Type material.** Holotype: Allan Hancock Foundation No.55 stored in Santa Barbara Museum of Natural History; collected from Hancock Foundation station, east of Portuguese Bend, California, United States. 33.71667° N, 118.33250° W, 14.6–13.7 m, bottom rocky, November 6 1949 (Figure 15). Colony encrusted upon hold-fast and blades of algae (_Halidrys_ sp.).

**Additional material.** NHMUK 1963.9.4.1. Pacific coast, North America, Charles H. O’Donoghue collection.

**Description.** Colonies cuticular, light to dark brown appearance, forming prominent incrustations (Figure 4). Colonies encrusting unilamellar, and/or erect, bilamellar back-to-back forming large colonies. Autozooids imperfectly rectangular to hexagonal, about 800 µm in length and 400 µm in width (Figures 4b, c, 12a). Peristome short but prominent. Vestibular wall distinctly folded (Figure 8c, f). Budding as multiple peripheral chambers that enlarge during ontogeny (Figure 10a). Pore complex of 0.02 mm diameter. Chitinous septum pierced by four minute perforations. Around 23 tentacles (Soule, 1951).

**Distribution.** Reported findings from the North Pacific are restricted to the Californian coast (United States) at Portuguese Bend, Santa Barbara-San Luis Obispo County line, Punta Baja and Pacific Grove, Monterey Bay (Soule in Osburn, 1953) (Figure 15). _Pherusella_

**FIGURE 7** Semithin sections through the cuticle of the frontal wall in pherusellids. The ectocyst has a thicker inner layer and a thinner outer layer. The thickness of the outer layer varies. Note the inclusions of the outer in the inner layer marked with an asterisk. (a) _Pherusella tubulosa_. (b) _P. flabellaris_. (c-d) _P. brevituba_. Note: Inclusions are different. (e) _P. minima_ with similar inclusions as in _P. tubulosa_. (f) Ancestrula of _P. minima_ with the remains of larval valves on the frontal wall. (g-h) _P. liowae_, note the strongly fringed ectocyst of the peristomial region. Abbreviations: bc—body cavity, ect—ectocyst, rlv—remains of larval valves.
FIGURE 8  Histological semithin sections through the aperture area along the longitudinal axis of all pherusellid species. (a) Pherusella tubulosa. (b) P. flabellaris (c) P. brevituba. (d) P. minima (e) P. liowae. (f) Detail of the cuticle in P. brevituba. (g) Detail of the cuticle in P. liowae. Abbreviations: bc—body cavity, c—collar, ect—ectocyst, o—orifice, pc—pore complex, t—tentacle, ts—tentacle sheath, v—vestibulum, vw—vestibular wall

| Pherusella | P. tubulosa | P. flabellaris | P. liowae | P. brevituba | P. minima |
|------------|-------------|---------------|-----------|--------------|-----------|
| Tentacle number | 30-33 | 28 | 24-26 | 23* | 19-21 |
| Pore number | 3-4 | 1 | 3-4 | 2-4* | 3 |
| Mean zood length (µm) | 1254 | 1132 | 887 | 872 | 805 |
| SD (n=10) | 111 | 119 | 49 | 62 | 61 |
| Min - max | 1038-1414 | 946-1334 | 776-958 | 766-950 | 710-997 |
| Mean zood width (µm) | 514 | 619 | 233 | 498 | 619 |
| SD (n=10) | 104 | 87 | 37 | 164 | 85 |
| Min - max | 391-708 | 432-764 | 185-325 | 447-630 | 511-995 |
| Peristome length (µm) | 824 | 762 | 340 | 266 | 201 |
| SD (n=10) | 97 | 91 | 41 | 21 | 22 |
| Min - max | 716-1024 | 606-910 | 283-395 | 230-300 | 172-231 |

* according to Soule, 1951

FIGURE 9  Summary of zooid dimensions, tentacle number and pore complexes of all pherusellid species. * according to Soule, 1951
brevituba has been reported for the Mediterranean Sea (Occhipinti-Ambrogi et al. 2011; Rosso & Di Martino, 2016; Zenetos et al. 2010) based on a single record by Chimenz Gusso and d’Hondt (2005), but its identity is questionable and probably belongs to another species (see below on *P. minima*).

**Remarks.** Soft-tissue data for this species are not included here as the analyzed material was not suitable and did not show properly preserved internal structures. The collection of fresh material was not possible. The species name derivates from the Latin *brevis*, short, alluding to the short tubular peristome compared to that in *P. tubulosa* and *P. flabellaris*.

### 3.7 | Pherusella minima sp. nov.

*Pherusella brevituba* Soule: Chimenz Gusso & d’Hondt, 2005 (p. 87, figure 2); Occhipinti-Ambrogi et al. 2011 (p. 223); Rosso & Di Martino, 2016 (p. 571).  
LSID: http://zoobank.org/urn:lsid:zoobank.org:act:C2D4F81B-6394-4877-A2EC-7142E53899A8

**Type material.** Holotype: ZSM-20200454, Bavarian State Collection of Zoology Munich, Northern Adriatic Sea, bay of Valovine in Pula, Istria, Croatia (44.85956° N, 13.81297° E) (Figures 6b, 15). Colony on a leaf of *Posidonia oceanica* at 2.5 m depth. Paratypes: ZSM-20200455, ZSM-20200456, ZSM-20200457, same locality as holotype.

**Additional material.** NHMUK 1975.1.12.331, stored in Natural History Museum London, collected at Chios, Greece in 1963, identified as *P. tubulosa*.

**Description.** Colonies always unilaminar, exclusively encrusting leaves of the seagrass *Posidonia oceanica*. Colonies small, rarely exceeding eight zooids (Figures 5a, 6b). Largest encountered colony 16 zooids. Colony biserial, opposing zooids facing away from their proximal neighbors, with their proximolateral walls mutually abutting. (Figures 5b; 10b). In young colonies zooids appear as flat oval disks; becoming irregularly rectangular to hexagonal in older colonies (Figures 5a, b; 6b, 10b). Zooids 805 µm in length and 619 µm in width, peristome short 201 µm in height (Figure 9). Ancestrula with remains of larval valves, ridges on the frontal wall about 300 µm in length and 250 µm in width (Figures 5b; 10b). Vestibular wall weakly folded. Collar about 50 µm in length (Figures 8d; 11d). Frontal duplicature bands broad with common base (Figures 5c, d). Apertural muscles as for the genus (Figures 5c, d; 12e). Lophophore with 19–21 tentacles (Figure 9). Tubular cardia, medium cecal pouch, anus vestibular. Several multiporous pore complexes (Figures 5c, d; 12e) with slender chitinous rim, septula with three perforations, each 1 µm in diameter (Figure 13g, h). Up to five embryos of different developmental stages within tentacle sheath (Figure 5b).

**Etymology.** From the Latin *minimus*, least, alluding to the consistently very small colonies, in contrast to the larger colonies of other members of the genus (particularly *Pherusella brevituba*), as well as to peristome height, which is the shortest within the genus.

**Distribution.** Specimens of *Pherusella minima* sp. nov. were found in 2018 and 2019 in the Northern Adriatic Sea along the Croatian coast, close to the city of Pula. Specimens were also reported in the western basin of the Mediterranean Sea at the French coast close to the city of Marseille in 2019. Investigation of material from historical collections (NHMUK 1975.1.12.331) indicates this species was already found in 1963 at Chios, Greece, encrusting *Posidonia oceanica* leaves (Figure 15). Similar specimens were reported from Sicily in 2004, also
exclusively encrusting the leaves of *P. oceanica* with small biserial colonies (Chimenz Gusso & d’Hondt, 2005). There are no reported findings outside of Mare Nostrum, suggesting *P. minima* sp. nov. is endemic for this geographical region (Decker et al. 2020 as *Pherusella* sp.).

**Remarks.** There are clear indications that *Pherusella minima* sp. nov. differs from *P. brevituba*. *P. minima* sp. nov. exclusively occurs on *Posidonia oceanica* and never forms large colonies, whereas *P. brevituba* grows on various substrates (predominantly algae) and regularly forms large colonies: *P. minima* sp. nov. might appear similar to *P. brevituba* in early ontogeny, but the formation of zooids differs in both species (see Figure 9). *P. brevituba* also forms peripheral chambers during its growth; these are lacking in *P. minima* sp. nov. Details in zooidal morphology such as tentacle number, pore-complex structure and vestibular wall morphology also differ (see also Figure 9).

3.8 | Key to pherusellid species

1. Zooids large, longer than 1000 µm and long peristome >700 µm ................................................................. 2

|   |   |
|---|---|
| 1. | Zooids small, no longer than 1000 µm and short peristome <400 µm ................................................................. 3 |
| 2. | 30 or more tentacles .................................................... *P. tubulosa* (Figure 1) |
| 3. | 28 tentacles ............................................................... *P. flabellaris* (Figure 2) |
| 4. | Peristome shorter than 230 µm and 19–21 tentacles ............................................................... *P. minima* sp. nov. (Figure 5) |
| 5. | Peristome longer than 230 µm ........................................... 4 |
| 6. | Zooids very narrow <400 µm and 24–26 tentacles ............................................................... *P. liowae* sp. nov. (Figure 3) |
| 7. | Zooids wider than 400 µm and 23 tentacles ............................................................... *P. brevituba* (Figure 4) |

4 | DISCUSSION

4.1 | Novel characters for old purposes: soft-tissue morphology and the necessity of histology

This study is the first to employ histological details on a comparative scale for revising a family of ctenostome bryozoans. Analyzing numerous details and characters such as digestive tract partitioning, presence
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and abundance of duplicature bands is not possible without classical histology and 3D-reconstruction techniques (see also Schwaha et al. 2019 for its use in taxonomic studies). These techniques become particularly useful if the zooidal cuticle is not transparent enough to recognize these characters externally. Other techniques such as confocal laser scanning microscopy yield good results for thin, transparent species such as *Pherusella minima*, recently studied in more detail (Decker et al. 2020 as *Pherusella* sp.), but remains of little use in thicker, bi- or multilaminar species. Dissection of single zooids is possible but will destroy and/or distort several morphological characters.

Emphasizing the importance of detailed morphology, it should be noted that many of the characters assigned in the current study for the diagnosis of the family Pherusellidae might be shared with closely related forms such as the Flustrellidridae. Owing to the lack of comparable datasets, similarly conducted studies on multiple ctenostome families would be helpful in order to address specific apomorphic characters. Examples for such characters include the multiporous interzooidal pore complexes and the bilateral lophophore with rejection tract shared with *Flustrellidra* and *Sundanella* (see Atkins, 1932; Braem, 1939, Schwaha, pers. observation). Ultimately, this will yield a clearer picture of ctenostome phylogeny, once a comparative database of multiple families will be available.

This study also shows the usefulness of old museum material for comparative morphological analyses. *Pherusella flabellaris* turned out to be an “easter egg” of preservation. Despite being collected prior to 1890, the type material shows exceptionally good preservation for histological purposes. This also enabled determination of its tentacle number from approximately 20 to 28. In contrast, the much younger specimens of *P. brevituba* were not suitable. Although storage of samples is usually in ethanol, the original preservation is often not documented.

4.2 | The distribution of pherusellids, an undiscovered field

As this review and survey shows, the geographic distribution of pherusellids is, with the exception of *Pherusella tubulosa*, sparse and little known. However, several previous reports, particularly of the latter species, have proven to be based on misidentifications (e.g., Woodward, 1833). Without careful examination, species such as *P. flabellaris* and

FIGURE 12  Schematic representation of pherusellids with retracted polypide from a basal perspective with main focus on the digestive tract, note the long cardia in all species and the cecal pouches of different sizes. Musculature is marked in red. (a) *Pherusella brevituba*, redrawn from Soule, 1951. (b) *P. tubulosa*. (c) *P. flabellaris*. (d) *P. liowae*. (e) *P. minima*. Abbreviations: bb—brown body, ca—cardia, cae—cecum, d—diaphragm, es—esophagus, int—intestine, l—lophophore, lv—larva, o—orifice, pc—pore complex, pd—parieto-diaphragmatic muscle, pm—parietal muscles, ph—pharynx, ps—peristome, py—pylorus, rm—retractor muscle, ts—tentacle sheath, v—vestibulum
**FIGURE 13** The pore complexes of all pherusellid species. Semithin sections on the left and the corresponding 3D reconstructions on the right. The pores are marked with an asterisk and the cuticular reinforcement of each complex in red. (a-b) *Pherusella tubulosa*. (c-d) *P. flabellaris*. (e-f) *P. brevituba*. (g-h) *P. minima*. Note the comparatively weak cuticular reinforcement. (i-j) *P. liowae*. Abbreviations: bc—body cavity, ect—ectocyst, pc—pore complex, spe—special cell

*P. liowae* sp. nov. might easily be misidentified as *P. tubulosa* (as might have been the case, e.g., in India—Rao & Ganapati, 1980). The geographic distribution of all pherusellid species seems to have specific limits, which is not surprising in species possessing lecithotrophic larvae which generally have a short dispersal range, and also settle shortly after their release (Reed, 1991). This is particularly puzzling in the wide distribution of *P. tubulosa* from Dominica to the Atlantic coast and Mediterranean Sea. The currently available data on tentacle number (see above) also underline the possibility of multiple species being present in the *P. tubulosa* complex, which, however, will require additional studies to confirm this. Some species can also show a high selectivity for the substratum, as first experimental studies on *P. minima* sp. nov. have shown that its larvae only settle on *Posidonia* leaves and not on any natural phytal substratum offered (Decker et al. 2020 as *Pherusella* sp.).

The lack of proper identifications and biodiversity studies can have drastic effects on the understanding of ecosystems as seen in the case of *P. minima* sp. nov. —presumably misidentified as *P. brevituba* in 2004. The current study shows that the epiphytic pherusellid restricted to *Posidonia oceanica* leaves is in fact a new species based on several differences like substrate specificity, morphological details, colony form, reproduction and astogeny. Based on the original distribution of *P. brevituba* from the Eastern Pacific (Soule, 1951), *P. minima* was most likely identified as *P. brevituba*. Based on a single assessment, *P. brevituba* has been temporarily regarded as alien species.
in the Mediterranean Sea (Chimenz Gusso et al. 2004; Occhipinti Ambrogi et al. 2011; Rosso & Di Martino, 2016). Although it would require study of the original material used as identification of *P. brevituba* in 2004 to confirm misidentification, the striking morphological and ontogenetical resemblance of the colonies to our observations on *P. minima* sp. nov. (see Chimenz Gusso & d’Hondt, 2005 (p. 87, figure 2)), indicate that *P. minima* sp. nov is most likely an endemic species to the Mediterranean Sea. One interesting discovery was that colonies collected from Chios, Greece, in the 1960s and labeled *P. tubulosa* confirm the presence of the species in the Mediterranean Sea 60 years ago, almost at the time of the *P. brevituba* description by Soule (1951).

Misidentification of specimens might result from assumptions that certain characters are only formed later in ontogeny. This has been shown for a closely related ctenostome: young colonies of *Flustrellidrella prouhoi* d’Hondt, 1974 were previously identified as young colonies of *Flustrellidra hispida* according to d’Hondt (1974). The former genus lacks spines, which, however, are diagnostic for the latter genus, which underlines that even young colonies already show important diagnostic characters. Hence there is also

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**FIGURE 14** Semithin section through the body cavity and lophophore of some pherussellids. (a) Cross section of the body cavity and polypide of *Pherusella flabellaris*. Holotype material (NHMUK 1889.8.21.128). (b) Longitudinal section of the body wall between two adjacent zooids of *P. tubulosa*. Note the prominent folds of the shared lateral body wall. (c) Cross section of the lophophore of *P. flabellaris*. Holotype material. Note 28 tentacles. (d) Longitudinal section of an embryo-brooding zooid of *P. flabellaris*. Three embryos develop within the tentacle sheath while the rest of the polypide is degenerated. Abbreviations: at—atrium, bc—body cavity, cae—cecum, e—embryo, ect—ectocyst, pm—pore complex, pm—parietal muscles, t—tentacle, tc—tentacle coelom, ts—tentacle sheath.
the possible association of *P. minima* sp. nov. colonies as young *P. tubulosa*, which, however, clearly differ in the size of the peristome (Figure 9).

5 | CONCLUSIONS AND OUTLOOK

This is the first study to analyze and revise an entire (admittedly small) family of ctenostomes with the aid of soft-body morphological information. It emphasizes that ctenostome identification requires histological sections for many of the small details that might otherwise be overlooked.

The essential piece missing for analyzing current species delimitations and relationships are molecular sequences, which for ctenostomes are still rare. Although successful sequences were obtained from two of the species that are geographically well separated (*P. minima* sp. nov. and *P. liowae* sp. nov.), our efforts to obtain fresh material of *P. tubulosa* and *P. brevituba* were not successful. Hence, future work on ctenostome taxonomy and biodiversity should aim for sequencing of more of these understudied taxa.

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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 available from the corresponding author upon reasonable request.

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