

Observations on *Hydractinia aculeata* (Hydrozoa, Cnidaria)

Peter SCHUCHERT

Muséum d'histoire naturelle, CP 6434, CH-1211 Genève 6, Switzerland.

E-mail: Peter.Schuchert@ville-ge.ch

Observations on *Hydractinia aculeata* (Hydrozoa, Cnidaria). - Two living colonies of the uncommon hydroid *Hydractinia aculeata* (Wagner, 1833) found in the Balearic Islands are described and depicted. The relationships to other similar hydractiniids producing medusoids are discussed. Partial sequences of the mitochondrial 16S RNA gene were determined in order to allocate the species to its correct genus within a recent classification scheme based on a molecular phylogeny of the family. The 16S sequences did not permit to associate the species to a known genus of this new classification because the lineage was rather distinct from all other genus-level clades. The species was thus left temporarily in the genus *Hydractinia*.

Keywords: Mediterranean - Hydractiniidae - hermit crabs - morphology - medusoid.

INTRODUCTION

Hydractinia aculeata (Wagner, 1833) is an endemic, uncommon Mediterranean hydroid living on shells of gastropods and hermit crabs. In spite of its well characterized morphology and recent re-description (Schuchert, 2008), modern illustrations are lacking. The present contribution therefore aims at illustrating the species based on living specimens, as well as discussing its relationships and taxonomy.

The generic subdivision of the family Hydractiniidae was traditionally based on the presence or absence of a medusa stage and the nature of the hydrorhiza in the polyp stage (e. g. Bouillon, 1985). Species with free medusae were placed in the genus *Podocoryna*. Polyp colonies with normal, reticulate hydrorhiza were assigned to the genus *Stylactaria*, while for those species in which the stolons coalesced into a crust-like hydrorhiza the genus *Hydractinia* was used. This classification scheme was then abandoned after the studies of Bouillon *et al.* (1997) and Boero *et al.* (1998) showed that these characters are unsuitable to distinguish genera because it is not possible to draw unambiguous separation lines between the genera. The three genera were thus united in the single genus *Hydractinia*. Recently, the family Hydractiniidae has again been revised, this time based on extensive molecular phylogenies (Miglietta *et al.*, 2009, 2010, 2012). In these studies, the genus *Hydractinia*, as defined by Boero *et al.* (1998) or Bouillon *et al.* (2006), was split again into several genera. Notably, the previously well-established genus *Podocoryna* was re-validated and comprises hydractiniids producing medusae or medusoids. Also the genus *Stylactaria* was re-validated,

but not based on the structure of the hydrorhiza, which was shown to be unsuitable for generic separations (but it may remain useful at the species level). The new classification is based entirely on DNA sequence data and many genera can in fact not be diagnosed using morphological characters alone. A similar situation is met with in at least another family of Anthoathecata, the Corynidae (Nawrocki *et al.*, 2010), suggesting that the problem is not unique to the Hydractiniidae. Such molecular based genus diagnoses may pose problems for allocating new species to their correct genus. Likewise, for species like *H. aculeata* that were not included in the original molecular analyses, it may be not evident to what genus they actually belong. Therefore, partial mitochondrial 16S RNA gene sequences obtained from the present material were compared to all known hydractiniid sequences in order to clarify its position within the new classification of the Hydractiniidae.

MATERIAL AND METHODS

Two colonies on hermit crabs were collected near Cala Murada, Mallorca, Balearic Islands (39.4521°N 3.2782°E) on 1st and 2nd July 2010, in a depth of 2 to 3 m. The shells were obtained by combing with an open hand through *Peyssonnelia* algae. The specimen MHNG:INVE:71904 is a male colony on a 12 mm long nassarid shell inhabited by a hermit crab. The specimen MHNG:INVE:71907 is a female colony growing on a 14 mm long shell of *Pisania striata* (Gmelin) inhabited by a hermit crab tentatively identified as *Cestopagurus timidus* (Roux) using Ingle (1993). Both colonies were kept alive for observation, then preserved in formalin, and subsequently deposited in the invertebrate collection of the Natural History Museum of Geneva.

DNA from a few polyps was extracted from both colonies and a fragment of the mitochondrial 16S RNA gene was amplified and sequenced as described in Schuchert (2005). The new sequences have been deposited in the EMBL database under the registration numbers HG976624 and HG976625. They were compared to the complete set of hydractiniid 16S sequences (GenBank PopSet 188474499) provided by Miglietta & Cunningham (2012) using maximum likelihood trees as described in Schuchert (2014). A subset of these 16S data, comprising only European species, was used similarly to examine whether *H. aculeata* is genetically distinct from the other European hydractiniids for which 16S sequences are known.

TAXONOMY AND RESULTS

Hydractinia aculeata (Wagner, 1833)

Figs 1-4

Hydra ovipara s. *aculeata* Wagner, 1833: 256, pl. 11 figs 1-10.

Coryne aculeata. – Ehrenberg, 1834: 294.

Hydra aculeata. – M. Sars, 1846: 8.

Hydractinia aculeata. – Allman, 1872: 352. – Motz-Kossowska, 1905: 86, fig. 10. – Herberts, 1964: 161. – Herberts, 1969: 351. – Bouillon *et al.*, 2004: 63, fig. 37A. – Schuchert, 2008: 245, fig. 9.

Stylactis aculeata. – Bavestrello, 1985: 351, fig. 2.

DIAGNOSIS: Colonies growing on gastropod shells, hydrorhiza encrusting or stolonal, gastrozooids 1.5-2 mm high, 12-14 tentacles in two close-set whorls, gonozooids smaller, 3-10 short tentacles, with mouth. Gonophores short-lived medusoids

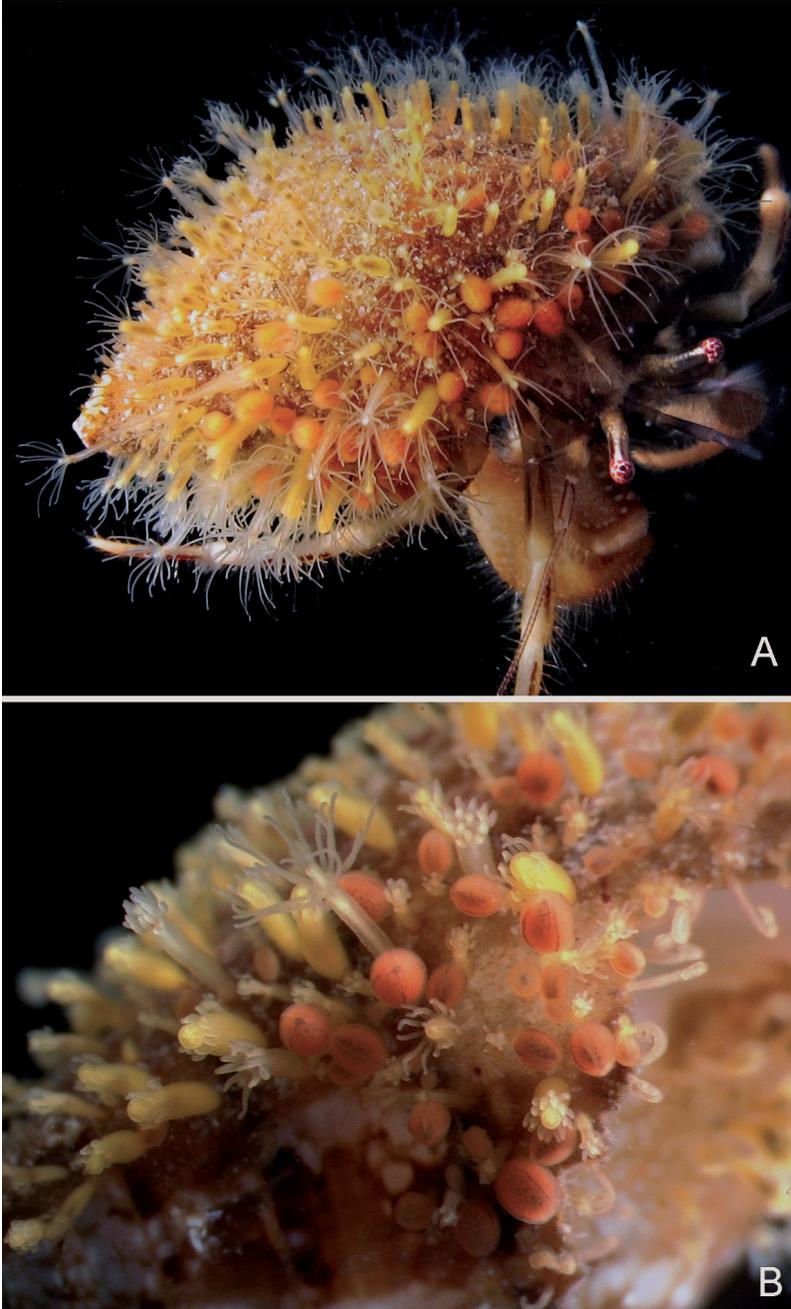


FIG. 1

Hydractinia aculeata, living colony from Mallorca. (A) Dorsal view of a hermit crab inhabited gastropod shell invested by hydroid colony (B) Portion of colony near shell aperture, showing gastro-, gono-, and tentaculozooids. The size of the shell is 14 mm.

with mature gonads, released or not, 20-36 eggs, no distinct tentacle bulbs, without or with 7-8 tentacle stumps.

DESCRIPTION OF EXAMINED MATERIAL: Colonies growing on different gastropod shells inhabited by the hermit crab *Cestopagurus timidus* (Roux) (Fig. 1). Hydrorhiza with varied structure, either stolonal (on the rugose nassarid shell) or forming a crust (on the smooth-walled shell); small spines present, smooth. Polyps polymorphic, comprising gastro-, gono-, and tentaculozooids. Colour of polyps yellow, female gonophores deep orange, male ones beige. All polyps without basal perisarc cup.

Gastrozooids 1.5-2 mm high when extended (Fig. 2B), with dome-shaped to slightly necked hypostome, covered by a dense band of contiguous nematocysts; 12-14 (rarely 18) tentacles in two close-set whorls. All tentacles held upwards, length differences less marked than in other hydractiniids.

Gonozooids resembling the gastrozooids and derived from them (Fig. 2D), comparatively smaller (ca. 1/3 their height), with mouth, and up to 10 shortened tentacles; in upper third of body one whorl of 1-3 medusa buds, buds globular to oblong, filled with gametes, radial canals and bulbs well visible. Mature, but still attached gonophores 0.8 mm long.

Tentaculozooids occur along the rim of the shell opening, slightly tapering, smaller than gastrozooids, coiling synchronously.

Gonophores short-lived medusoids that can be released (Fig. 2E) or that remain attached (Fig. 3). Liberated medusoids rather inactive, spherical to oblong, jelly moderately thick and even, exumbrella without nematocysts, velum present, no distinct perradial bulbs, with 7-8 tentacle stumps. Manubrium spanning about half the sub-umbrellar height, without mouth, surrounded by a very thick gonad without perradial interruptions, females with 30-36 eggs. Colonies dioecious.

Nematocysts:

- large microbasic heteronemes (Fig 4C-D) on hypostome, with faint swelling of shaft, size of native capsules $(3.5-4.5) \times (10-10.5) \mu\text{m}$; the shaft in discharged capsules is variable and may appear either as characteristic for euryteles or for mastigophores;
- smaller microbasic euryteles on tentacles and body (Fig. 4B), size of native capsules $(3-3.5) \times (7-8) \mu\text{m}$;
- desmonemes on tentacles and body (Fig. 4A), size of native capsules $(3.5-4) \times (6-7) \mu\text{m}$.

BIOLOGY: Grows on various gastropod shells, inhabited either by molluscs or hermit crabs. For more details see Schuchert (2008).

DISTRIBUTION: Mediterranean. Type locality: Trieste, Adriatic Sea.

PHYLOGENY: The two 16S sequences (583 base pairs long) obtained from the present material differ in only two positions. They were compared to the 16S sequences published by Miglietta & Cunningham (2012). A phylogenetic tree based on maximum likelihood analysis (HKY model, 100 bootstrap pseudoreplicates, not shown) did not associate *H. aculeata* with sufficient bootstrap support to any other species or clade. It is, however, well delimited from all other European species (Fig. 5).

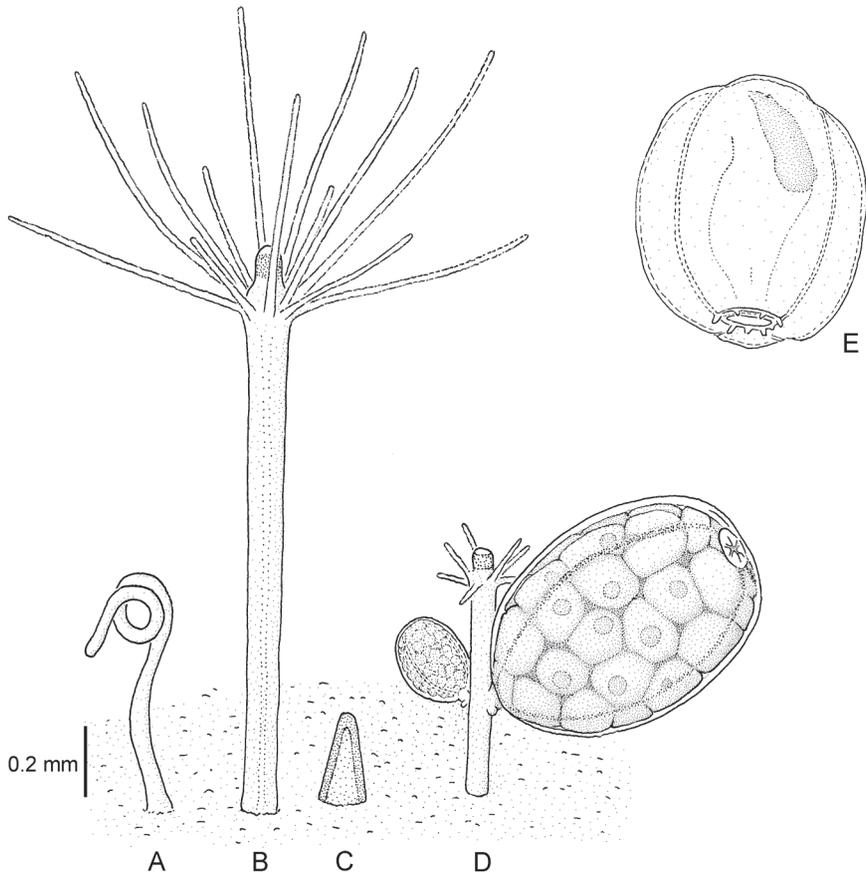


FIG. 2

Hydractinia aculeata from Mallorca, after life, all structures or polyps drawn to the same scale. (A) Tentaculozoid. (B) Gastrozoid. (C) Spine. (D) Gonozoid with incipient (left) and mature (right) medusoids. (E) Released medusoid after spawning, note rudimentary tentacles.

DISCUSSION

The morphological and molecular data provided herein are intended to allow an easier, more reliable identification of *H. aculeata*. Although the description of Wagner (1833) is surprisingly precise and complete for its time (see reproduction of his figures in Schuchert, 2008), the species remains rather difficult to separate from a number of nominal taxa occurring in the Mediterranean and along the European Atlantic coast. Other medusoids producing *Hydractinia* species which have been reported in European waters are: *H. allmani*, *H. calderi*, *H. hooperii*, *H. inermis*, *H. pruvoti*, and *H. proboscidea* (comp. Schuchert, 2008). The most important distinguishing characters are the distribution and the type of substrate (Table 1).

TABLE 1: Taxonomic characters used to separate medusoid-producing hydractinids reported in European waters. Data are from Sigerfoos (1899), Nutting (1901), Bouillon (1971), and Schuchert (2008). The table is likely not exhaustive and more differences may exist. Many descriptions are not really comparable as they are often based on preserved material only.

species character	<i>allmani</i>	<i>calderi</i>	<i>hooperii</i> (not <i>arge</i>)	<i>inermis</i>	<i>proboscidea</i> (not <i>calderi</i>)	<i>pruvoti</i>	<i>aculeata</i>
distribution	Arctic Sea, northern Norway	western Mediterranean	western Atlantic	Mediterranean	north-eastern Atlantic	Mediterranean	Mediterranean
hydrorhiza	coalesced into crust, naked coenosarc on top	reticulate stolons	reticulate stolons	reticulate stolons	reticulate stolons	reticulate stolons or coalesced into crust	reticulate stolons or coalesced into crust
substrate	gastropods	gastropods	gastropods	algae and other fixed substrates	rock, laminarian holdfasts	gastropod shells inhabited by molluscs or hermit crabs	gastropod shells inhabited by molluscs or hermit crabs
spines	present	present	present	absent	absent	small or absent	present
gastrozooid size [mm]	5-15	1-5	up to 25	1.5-6	up to 16	1-6	1.5-5
tentacles of gastrozooids	10-16	20-40 in up to 3 whorls	up to 20 in one whorl	16-18 (max. range 12-22)	14-22 (max. range 10-32)	8-14 in 2 whorls	8-12 (max. range 5-18)
tentacles of gonozooids	usually 4, range 0-6	2-14	6-8	6-16	6-12	1-2	3-7
band of nematocysts on hypostome	yes	yes	?	yes	yes	no	yes
egg number per gonophore	up to 100	>20	>30	10-20	16-70	>100	30-36

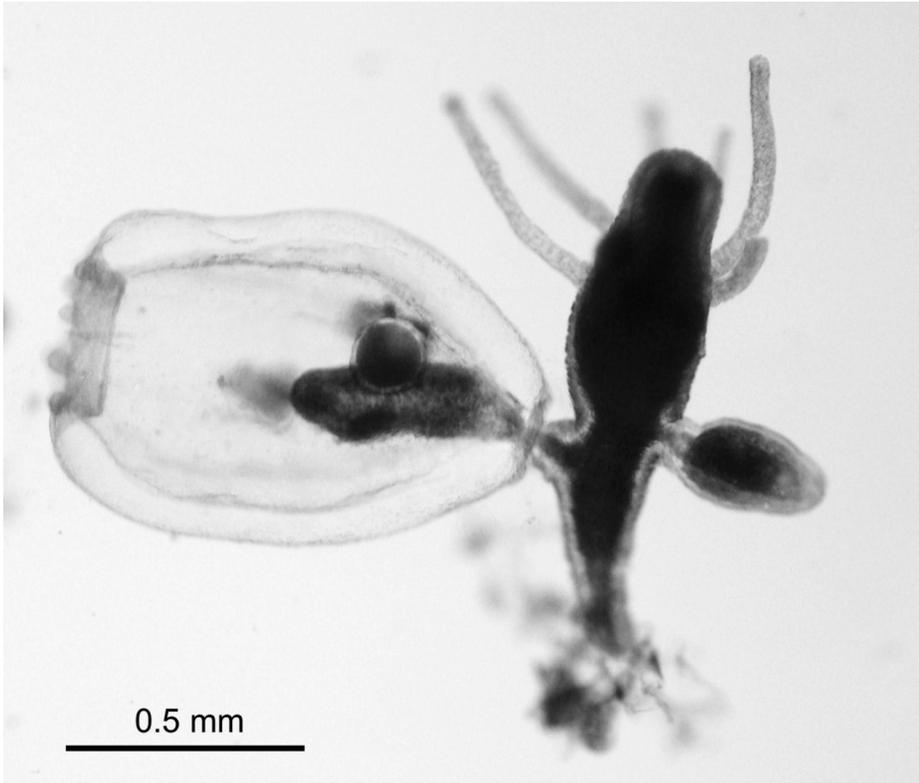


FIG. 3

Hydractinia aculeata, photo of an isolated gonozooid with attached medusoid after spawning (left), and incipient gonophore (right).

Hydractinia almani Bonnevie, 1898 (now *Schuchertinia allmani*) is an arctic to northern boreal species with much larger hydranths than *H. aculeata*. The 16S sequence data show that it is distinct from *H. aculeata* (Fig. 5).

Hydractinia calderi Bouillon, Medel, & Peña Cantero, 1997 (now *Bouillonactinia calderi*) was regarded as conspecific with *H. proboscidea* in my earlier (2008) publication. However, *H. proboscidea* is only known to occur on immobile substrata (see below), this in contradistinction to *B. calderi* which grows on gastropod shells only. *Bouillonactinia calderi* has large (5 mm) polyps with numerous tentacles (up to 40), which is significantly higher than noted for *H. aculeata*. Mediterranean material tentatively identified as *B. cf. calderi* in the study of Miglietta & Cunningham (2012) proved to be distinct from *H. aculeata* at the molecular level (Fig. 5). New, living animals and 16S data must be obtained of topotypical *B. calderi* material for a more conclusive evaluation of its identity.

Hydractinia hooperii (Siegerfoos, 1899) (now *Bouillonactinia hooperii*) was recorded once from the Mediterranean by Peña Cantero & García Carrascosa (2002). This is an American species and Schuchert (2008) assigned, with some reservation, the

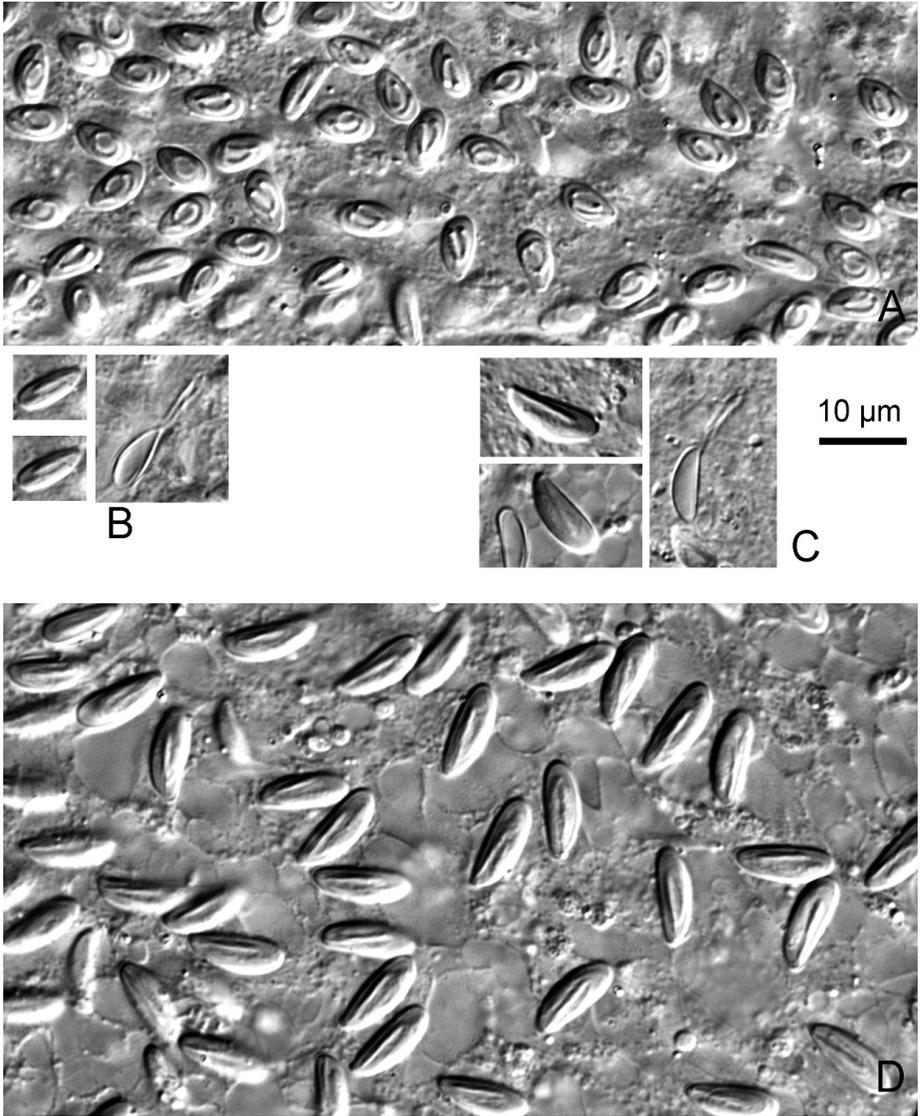


FIG. 4

Hydractinia aculeata from Mallorca, cnidome. (A) Desmonemes and small microbasic euryteles from tentacles. (B) Small microbasic euryteles; left undischarged, right discharged. (C) Large microbasic heteroneme from hypostome; left undischarged, right discharged. (D) Large microbasic heteronemes from hypostome.

Mediterranean record to *H. proboscidea*. According to the new data obtained here, it seems more likely that the Mediterranean records of *B. hooperi* belong actually to *H. aculeata*. The 16S sequences of the American *H. hooperi* were clearly distinct from the ones of *H. aculeata* (Fig. 5).

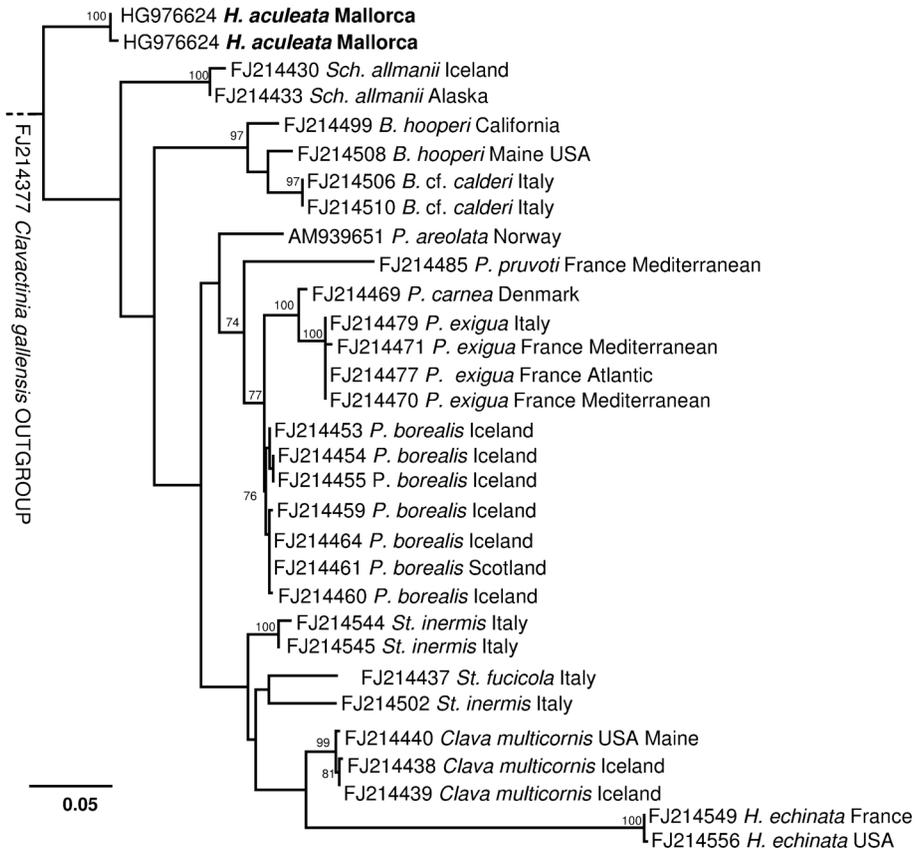


FIG. 5

Molecular phylogeny of NE Atlantic and Mediterranean hydractiniids based on partial 16S sequences: maximum likelihood tree obtained with PhyML (HKY model), 100 bootstrap pseudo-replicates, node support values only given if >70%. The tree was rooted using an outgroup, but the outgroup branch is shown shortened for clarity reasons. The numbers preceding the species names are the EMBL/Genbank accession numbers of the corresponding sequences.

Hydractinia inermis (Allman, 1872) (now *Stylactaria inermis*) can release medusoids with eight rudimentary tentacles, although the medusoids are sometimes retained on the gonozooids (pers. comm. Ivona Onofri, Dubrovnik). The species differs from *H. aculeata* in its substrate specificity, as it occurs only on non-mobile substrata like algae, sponges, rocks, barnacles etc. It also lacks spines and the hydrorhiza is never encrusting. The 16S data confirmed that it is distinct from *H. aculeata* (Fig. 5).

Hydractinia proboscidea (Hincks, 1868) is a very rare species only known from the Atlantic coast of Europe. It resembles closely *H. aculeata* but occurs on non-mobile substrata, like rocks and laminarian holdfasts. It differs additionally in having a stolonal hydrorhiza and the lack of spines. As for *H. calderi*, new, living material and 16S data must be obtained for a more conclusive taxonomic evaluation of this species.

Hydractinia pruvoti Motz-Kossowska, 1905 (now *Podocoryna pruvoti*) is easily separable as its gonozooids have one, occasionally two, tentacles only. The 16S data confirmed that it is distinct from *H. aculeata* (Fig. 5).

The 16S sequence data did not permit to assign *H. aculeata* reliably to a genus of the new classification introduced by Miglietta & Cunningham (2012). The 16S sequence alone is insufficient to resolve the genus clades (see Miglietta *et al.*, 2009). *Hydractinia aculeata* did also not cluster with convincing support with any of the other analyzed species, which would have allowed assigning it to the same genus. Additional, nuclear gene sequences are thus necessary to solve this issue. However, determining complete 18S and 28S gene sequences is much more costly than 16S sequences and may be beyond the capabilities of many institutions. This makes the new system rather cumbersome for all species which were not included in the original analyses. Likewise, it may prove to be difficult for some newly discovered hydractiniid species to assign them to the correct genus in the absence of 18S and 28S sequence information.

For the time being, *Hydractinia aculeata* is therefore left in the genus *Hydractinia*, a taxon which should be used as the default genus for all hydractiniids with unresolved genus affiliation.

The sequence data can also be used to examine if they permit to delimit *H. aculeata* from other European hydractiniids using a barcoding approach (Hebert *et al.*, 2003). The resulting tree (Fig. 5) confirms that the present samples of *H. aculeata* are easily separable from *Sch. allmani*, *B. calderi*, *B. hooperi*, *P. pruvoti* and *St. inermis*.

REFERENCES

- ALLMAN, G. J. 1872. A monograph of the gymnoblastic or tubularian hydroids. Conclusion of Part I, and Part II, containing descriptions of the genera and species of Gymnoblastea. *Ray Society, London*, pp. 155-450, plates 1-23.
- BAVESTRELLO, G. 1985. Idroidi simbiotici di paguri e gasteropodi nella riviera Ligure di Levante. *Oebalia* 11: 349-362.
- BOERO, F., BOUILLON, J. & PIRAINO, S. 1998. Heterochrony, generic distinction and phylogeny in the family Hydractiniidae (Hydrozoa: Cnidaria). *Zoologische Verhandelingen, Leiden* 323: 25-36.
- BONNEVIE, K. 1898. Zur Systematik der Hydroiden. *Zeitschrift für Wissenschaftliche Zoologie* 63: 465-495, plates 25-27.
- BOUILLON, J. 1971. Sur quelques hydroïdes de Roscoff. *Cahiers de Biologie Marine* 12: 323-364.
- BOUILLON, J. 1985. Essai de classification des hydropolypes-hydroméduses (Hydrozoa-Cnidaria). *Indo-Malayan Zoology* 2: 29-243.
- BOUILLON, J., GRAVILI, C., PAGES, F., GILI, J. M. & BOERO, F. 2006. An introduction to Hydrozoa. *Mémoires du Muséum National d'Histoire Naturelle* 194: 1-591.
- BOUILLON, J., MEDEL, M. D., PAGES, F., GILI, J. M., BOERO, B. & GRAVILI, C. 2004. Fauna of the Mediterranean Hydrozoa. *Scientia Marina* 68 (Suppl. 2): 1-448.
- BOUILLON, J., MEDEL, D. & PEÑA CANTERO, A. L. 1997. The taxonomic status of the genus *Stylactaria* Stechow, 1921 (Hydroidomedusae, Anthomedusae, Hydractiniidae), with the description of a new species. *Scientia Marina* 61: 471-486.
- EHRENBERG, C. G. 1834. Beiträge zur physiologischen Kenntniss der Corallenthiere im allgemeinen, und besonders des rothen Meeres, nebst einem Versuche zur physiologischen Systematik derselben. *Abhandlungen der Königlichen Akademie der Wissenschaften, Berlin* 1: 225-380.

- HEBERT, P. D. N., CYWINSKA, A., BALL, S. L., DE WAARD, J. R., 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London [Biology]* 270: 313-321.
- HERBERTS, C. 1964. Note au sujet de la reproduction de l'hydraire *Hydractinia aculeata* (Wagner, 1833). *Recueil des travaux de la Station marine d'Endoume fascicule 50* (bulletin 34): 161-165.
- HERBERTS, C. 1969. Note au sujet du développement de *Nassa pygmaea* Lamarck et *Hydractinia aculeata* (Wagner) 1833. *Recueil des travaux de la Station marine d'Endoume fascicule 61* (bulletin 45): 351-357.
- HINCKS, T. 1868. A history of the British hydroid zoophytes. *John van Voorst, London*, pp. Volume 1: i-lxvii + 1-338, volume 2, pls 1-67.
- INGLE, R. W. 1993. Hermit crabs of the northeastern Atlantic Ocean and Mediterranean Sea: an illustrated key. *London & New York, Chapman & Hall*, pp. 495.
- SARS, M. 1846. Fauna littoralis Norvegiae, I Heft: Über die Fortpflanzungsweise der Polypen. *Johann Dahl, Christiania*, pp. 94, plates.
- MIGLIETTA, M. P. & CUNNINGHAM, C. W. 2012. Evolution of life cycle, colony morphology, and host specificity in the family Hydractiniidae (Hydrozoa, Cnidaria). *Evolution* 66: 3876-3901.
- MIGLIETTA, M. P., MCNALLY, L. & CUNNINGHAM, C. W. 2010. Evolution of Calcium Carbonate skeletons in the Hydractiniidae. *Integrative and Comparative Biology* 50: 428-435.
- MIGLIETTA, M. P., SCHUCHERT, P. & CUNNINGHAM, C. W. 2009. Reconciling genealogical and morphological species in a worldwide study of the family Hydractiniidae (Cnidaria, Hydrozoa). *Zoologica Scripta* 38: 403-430.
- MOTZ-KOSSOWSKA, S. 1905. Contribution à la connaissance des hydraires de la Méditerranée occidentale. I. Hydraires gymnoblastiques. *Archives de Zoologie Expérimentale et générale, 4ème série* 3: 39-98.
- NAWROCKI, A. M., SCHUCHERT, P. & CARTWRIGHT, P. 2010. Phylogenetics and evolution of Capitata (Cnidaria: Hydrozoa), and the systematics of Corynidae. *Zoologica Scripta* 39: 290-304.
- NUTTING, C. C. 1901. The Hydroids of the Woods Hole region. *Bulletin of the U. S. Fish Commission for 1899* 19: 325-386.
- PEÑA CANTERO, A. L. & GARCÍA CARRASCOSA, A. M. 2002. The benthic hydroid fauna of the Chafarinas Islands (Alboran Sea, western Mediterranean). *Zoologische Verhandelingen* 337: 1-180.
- SCHUCHERT, P. 2005. Species boundaries in the hydrozoan genus *Coryne*. *Molecular Phylogenetics and Evolution* 36: 194-199.
- SCHUCHERT, P. 2008. The European athecate hydroids and their medusae (Hydrozoa, Cnidaria): Filifera Part 3. *Revue suisse de Zoologie* 115: 221-302.
- SCHUCHERT, P. 2014. High genetic diversity in the hydroid *Plumularia setacea*: A multitude of cryptic species or extensive population subdivision? *Molecular Phylogenetics and Evolution* 76: 1-9.
- SIGERFOOS, C. P. 1899. A new hydroid from Long Island Sound. *American Naturalist* 33: 801-807.
- WAGNER, R. 1833. Über eine neue im adriatischen Meere gefundene Art von nackten Armpolypen und seine eigenthümliche Fortpflanzungsweise. *Isis oder encyclopädische Zeitung* 1833: 256-260.

