

SHORT COMMUNICATION

# On the presence of *Digenea simplex* (Wulfen) C. Agardh (Rhodomelaceae, Ceramiales) in the northern Adriatic

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**Abstract:** During a macroalgal survey along the Istrian coast in the northern Adriatic, a population of *Digenea simplex* was observed near the coastal town of Funtana. Additional specimens were observed and collected at four nearby locations, including areas close to Poreč, Vrsar and Rovinj. According to the available literature, the species is stated as present but is considered rare in the northern Adriatic, with no published records of the species since late 19<sup>th</sup> and early 20<sup>th</sup> century. Here we present the recently located *D. simplex* specimens and settlements on the western Istrian coast, relatively close to the species' *locus typicus*, along with a historical overview of its presence in the northern Adriatic and beyond.

**Keywords:** *Digenea simplex*; northern Adriatic; western Istrian coast; historical overview

**Sažetak:** O PRISUSTVU VRSTE *DIGENEA SIMPLEX* (WULFEN) C. AGARDH (RHODOMELACEAE, CERAMIALES) U SJEVERNOM JADRANU. Tijekom istraživanja makroalgi, naselje vrste *Digenea simplex* otkriveno je u blizini Funtane na zapadnoj obali Istre, u sjevernom Jadranu. Primjerci su također opaženi i prikupljeni na dodatne četiri lokacije, u blizini Poreča, Vrsara i Rovinja. U dostupnoj literaturi stoji kako je vrsta prisutna, ali se smatra rijetkom u sjevernom Jadranu, bez jednog pronađenog primjerka od kraja 19. i početka 20. stoljeća. U ovom istraživanju predstavljamo nedavni pronalazak jedinki i naselja vrste *D. simplex* na zapadnoj obali Istre, u relativnoj blizini originalnog lokaliteta nalaza ove vrste (*locus typicus*). Ujedno dajemo i povijesni pregled dosadašnjih poznatih nalaza ove vrste u sjevernom Jadranu i šire.

**Gljučne riječi:** *Digenea simplex*; sjeverni Jadran; zapadna obala Istre; povijesni pregled

## INTRODUCTION

*Digenea simplex* (Wulfen) C. Agardh (Rhodomelaceae, Ceramiales) was first described in Gulf of Trieste in 1803 as *Conferva simplex* (Agardh, 1822; de Wulfen, 1803). Although it is considered pantropically distributed (Guiry and Guiry, 2024), it has been reported from temperate seas worldwide, including the Mediterranean (Agardh, 1822; Orfanidis and Breeman, 1999) and the Adriatic (de Wulfen, 1803; Agardh, 1822; Naccari, 1928; Hauck, 1885; Thomé and Migula, 1909; Vatova, 1928, 1948; Ercegović, 1966, 1980; Špan, 1980). Its northernmost occurrence appears to be in the northern Adriatic (Giaccone, 1978; Guiry and Guiry, 2024), where it is considered rare.

This species of red algae is recognised by a red, rigid, cartilaginous, hairy thallus, around 4–20 cm in height, depending on the source and the growth locality (Hauck, 1885; Thomé and Migula, 1909; Rodríguez-Prieto *et al.*, 2013). The thallus is affixed to the substrate by a basal disk and consists of indeterminate axes (branches), 1–1.5 mm thick, that are semi-dichotomously or irregularly branched (Thomé and Migula, 1909; Rodríguez-Prieto *et al.*, 2013; Schneider *et al.*, 2018). Indeterminate axes are covered by hair-like determinate axes of a varying

shade of red, occasionally forming small tufts on the apices, likely due to seasonal loss or grazing (Thomé and Migula, 1909; Rodríguez-Prieto *et al.*, 2013; Schneider *et al.*, 2018). The species has a three-phase life cycle with isomorphic gametophyte and sporophyte generations (Rodríguez-Prieto *et al.*, 2013; Schneider *et al.*, 2018) and reaches fertility during the August, according to literature data (Vatova, 1948). It is a perennial, epilithic algae typically found on rocky substrates in the infralittoral zone but can also tolerate environments with significant accumulation of particulate matter, such as mud and sand (Rodríguez-Prieto *et al.*, 2013; Schneider *et al.*, 2018).

The aim of this study was to map the distribution of recently located *D. simplex* with a focus on the settlement near the town of Funtana on the western Istrian coast (northern Adriatic) and to place this finding within a broader historical framework. This work reports the presence of *D. simplex*, a rare species in the northern Adriatic after more than a century.

## MATERIALS AND METHODS

During multiple phycological surveys, conducted from 2020 to 2022 and aimed at mapping of macroalgal

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communities along the different sections of the Istrian coastline, *Digenea simplex* was identified at several locations. Firstly, washed-up specimens were observed, followed by isolated thalli and, finally, two whole settlements along the western Istrian coast were found. Specimens were collected for identification, further observation *in vivo*, as well as preservation in herbarium and formalin. Collected samples of the thallus were compared with herbarium specimens collected by Hauck in the late 19<sup>th</sup> century and provided by Schneider *et al.* (2018) in his work. Samples were also dissected in order to confirm the identity of the species based on morphological characteristics according to Schneider *et al.* (2018) and Rodríguez-Prieto *et al.* (2013). Dissection was performed by hand and razor. Samples were microscopically observed and photographed with digital cameras. Cell measurements were conducted using ImageJ software.

The settlement of *D. simplex* we focused on is located in a small bay just outside the town of Funtana on the western Istrian coast, Croatia (Fig. 1). An assessment of this settlement was performed *via* snorkelling to determine the spatial distribution and population density of *D. simplex*. Distribution and settlement size were measured using a tape and the average population density was assessed by counting specimens within ten 40 x 40 cm randomly placed quadrats.

Afterwards, historical distribution data of *D. simplex* in the Adriatic Sea was compiled and analysed from multiple sources including online herbarium repositories, physical herbaria specimens, and older (19<sup>th</sup> and early 20<sup>th</sup> century), as well as more contemporary historical biodiversity publications (Table 1, Table S1 in Supplementary Material).

## RESULTS AND DISCUSSION

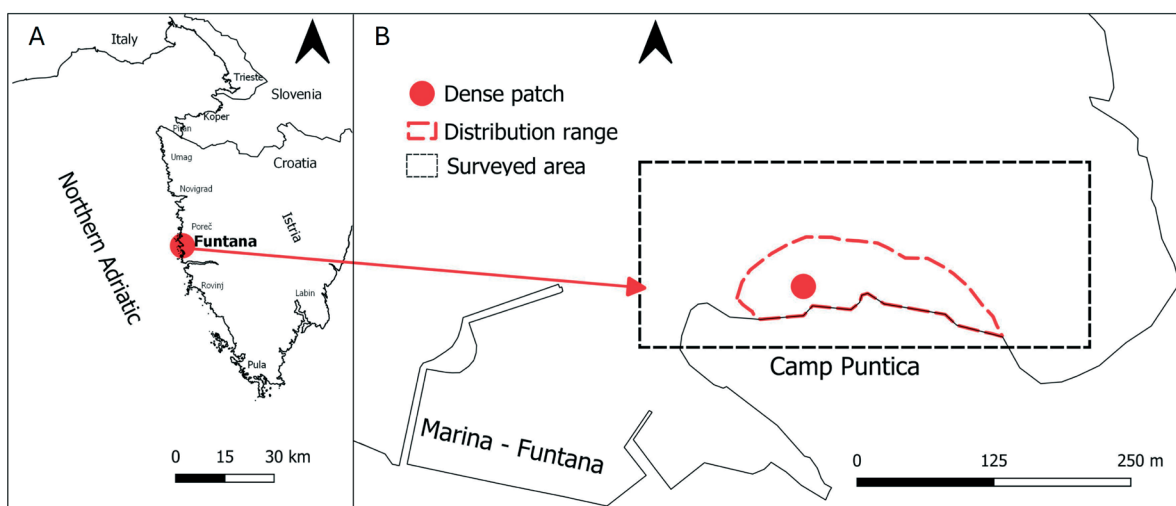
Specimens of *Digenea simplex* were first found washed up in Pical – Poreč (45.231815° N, 13.598529°

E) in December 2020 and Plava laguna – Poreč (45.213507° N, 13.593277° E) in March 2021 (Fig. S1A and S1B in Supplementary Material). Afterwards, in July 2021, a single thallus was also observed in Cape Muntravo – Rovinj (45.070294° N, 13.626067° E) in the subtidal zone (Fig. 2).

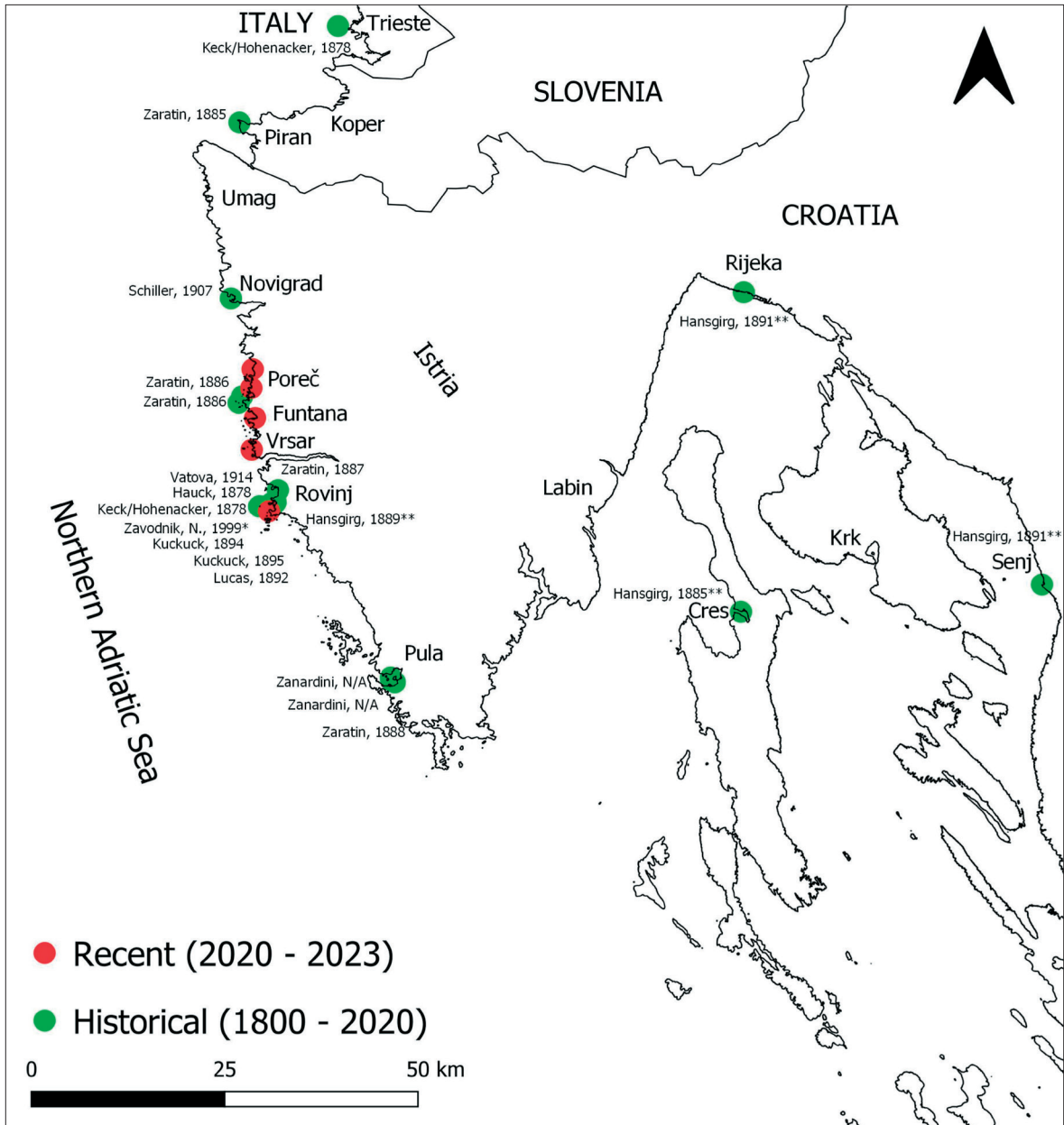
The settlement in Funtana (45.178481° N, 13.600051° E) was detected during March 2021 and surveyed during October 2021 (Fig. 1) at the depth of 0-1 m. *Digenea simplex* thalli were found attached either to larger cobbles, or affixed to a rocky substrate. This settlement was part of a somewhat degraded photophilic community, associated with species such as *Padina pavonica* (Linnaeus) Thivy, *Caulerpa cylindracea* Sonder, *Wrangelia penicillata* (C. Agardh) C. Agardh, *Anadyomene stellata* (Wulfen) C. Agardh, *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, *Cystoseira foeniculacea* (Linnaeus) Greville, *Dasycladus vermicularis* (Scopoli) Krasser, and *Rytiphlaea tinctoria* (Clemente) C. Agardh. The thalli were initially difficult to discern due to heavy sediment coverage (Fig. 3A) and epibionts (Fig. 3B). Recruits were also seen on rocks bearing the adult thalli in Funtana, resembling adults but with shorter axes and no branches (Fig. 3C, 3D).

The surveyed settlement in Funtana was unevenly distributed over an area of approximately 5300 m<sup>2</sup>. Within the area of distribution, *D. simplex* formed one large patch with continuous and dense algal distribution, covering about 20 m<sup>2</sup> with an average density of 57.5 thalli/m<sup>2</sup>. The remainder of the area of presence was sparsely settled, with only occasional isolated thalli (Fig. 1B).

The collected thalli from the location near Funtana (Fig. 3) fully resembled both recently washed-up specimens and older historical herbarium specimens from Rovinj (Fig. S1 in Supplementary Material; Battelli and Alberti, 2003; Schneider *et al.*, 2018). Transverse dissections of the indeterminate axis below the apex



**Fig. 1.** A cartographic representation of studied area in the northern Adriatic (A) and mapped *Digenea simplex* distribution (B). The local map was created in QGIS 3.16 Hannover.

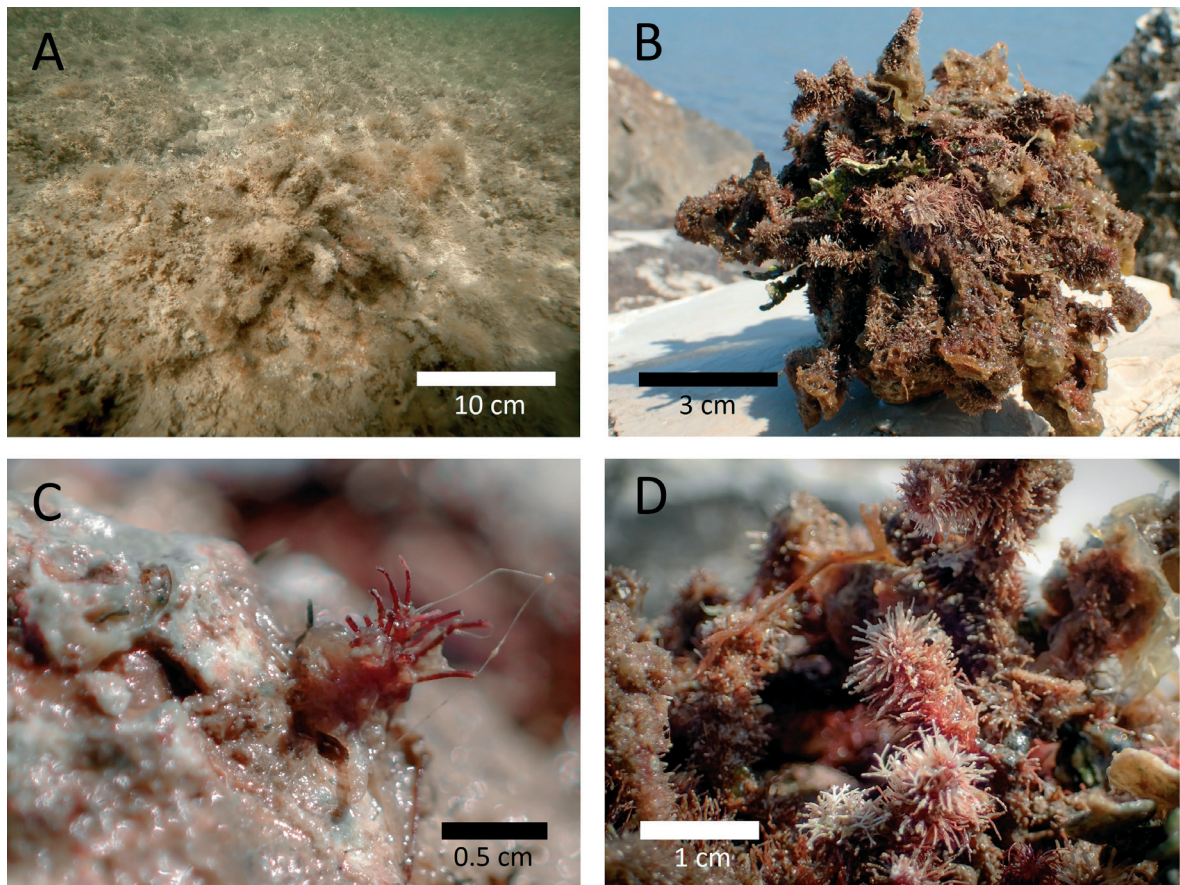


**Fig. 2.** Historical and recent collections of *Digenea simplex* centred around the Croatian part of the northern Adriatic. Green dots represent the historical findings of *D. simplex*, with the associated record name and year and red dots represent recent findings. Records mentioned in Table 1 that are outside the boundaries of the northern Adriatic are not shown. A recent *D. simplex* sample misplaced in the herbarium is marked with an asterisk (\*). Misidentified samples by Hansgirg are marked with a double asterisk (\*\*). Map created in QGIS 3.16 Hannover.

(Fig. S2A in Supplementary Material) revealed a tightly packed cell mass surrounded by an outer layer with radially growing determinate axes, which is indicative of this species. Transverse dissection of determinate axes (Fig. S2B in Supplementary Material) showed a pericentral cell layer (9 cells, 32–39  $\mu\text{m}$  diameter) around an axial cell (61  $\mu\text{m}$  diameter) and a single outer cell layer (28 cells, 24–40  $\mu\text{m}$  diameter). Similar characteristics were also observed in a dissected preserved specimen from Rovinj collected in the late 19<sup>th</sup> century by Hauck (Schneider *et al.*, 2018).

In June 2022, another settlement was located near Petalon – Vrsar (45.140586° N, 13.596163° E) at the depth of 5 m, in this case within a well-developed photophilic community, associated with species like *Dicthyota dichotoma* (Hudson) J.V. Lamouroux, *P. pavonica*, *Aplysina aerophoba* (Nardo, 1833), *Flabellia petiolata* (Turra) Nizamuddin, *Gongolaria montagnei* (J. Agardh) Kuntze, and *Ericaria corniculata* (Turner) Zanardini. The thalli here were more easily recognisable despite supporting noticeable epibiota, as well as due a lack of





**Fig. 3.** A collected specimen of *Digenea simplex*: in-situ, as observed (A); a collected and cleaned specimen (B); a recruit-stage individual (C); a close-up photo of the thallus showing cartilaginous indeterminate and hair-like determinate branches (D).

sediment cover owing to increased wave exposure in the area.

Two additional preserved specimens from 1999 collected close to town of Rovinj were uncovered in the herbarium collection in the Center for Marine Research, Rovinj (Fig. S3 in Supplementary Material). These specimens were misidentified as *Cystoseira* sp. and misplaced among brown macroalgae, a situation which, if common, may hide even more samples among herbaria, a case likely not limited only to this species.

According to literature, the presence of *D. simplex* in the Adriatic Sea can be traced to the early 19<sup>th</sup> century, with de Wulfen (1803) describing the species and its occurrence in the Adriatic. Going forward, Agardh (1820) states that the species is found in the Mediterranean and the Adriatic, on the coasts of Italy, Liburnia and Liguria. Afterwards, Naccari (1828) provides the information that the species grows in the area of Trieste, above the larger marine flora. Interestingly, he cites de Wulfen (1803) as the source of this information, suggesting the potential area of observation. Following up on that, Hauck provides a description of the species' habitus in his texts but refers to its distribution only as "in the Adriatic Sea" (Hauck, 1885). This is (although vague) supported by, at the time, older scientific sources

(de Wulfen, 1803; Agardh, 1820; Naccari, 1828), as well as contemporary and later herbarium collections (Algae Herbarium Portal; Table 1). In the 1960s, Ercegović (1960, 1966) classified *D. simplex*, noted prior to, and collected during the 1960's in the central Adriatic, as a relict, pantropical species from the Tethys era, suggesting its rarity in the "cold" Adriatic.

While a number of *D. simplex* samples can be found in locally available herbaria, others are either lost or held in private or museum collections abroad, with some available online as herbarium scans/photos (Munda 2000; Battelli and Alberti, 2003; Algae Herbarium Portal; GBIF; Paris NHM) (Table 1). Munda (2000) documented algological records from various collectors, showing long-term changes in the marine flora. *Digenea simplex* was documented near Rovinj in 1892 by Lucas and in 1894-95 by Kuckuck, but it was notably absent in subsequent surveys conducted during the 1960s, 1970s and 1980s (Munda, 2000). Afterwards, Battelli and Alberti (2003) compiled herbarium samples collected by Zaratini in the late 19<sup>th</sup> century along the western Istrian coast (Table 1, Fig. 2), which included some of the *D. simplex* samples. In addition, while presumably collected in the same period, some older Hauck's samples from Rovinj remain undated (Algae Herbarium Portal). Also

**Table 1.** Locations where *Digenea simplex* was collected or noted on the eastern Adriatic coast in the 19<sup>th</sup> and early 20<sup>th</sup> century. Sources are represented by numbers: 1 – de Wulfen, 1803; 2 – Naccari, 1828; 3 – Algae Herbarium Portal; 4 – Muséum national d'Histoire naturelle, Paris, France Collection: Cryptogams (PC); 5 – Battelli and Alberti, 2003; 6 – Munda, 2000; 7 – Vatova, 1928; 8 – Ercegović, 1980; 9 – Ercegović, 1966; 10 – Špan, 1980; 11 – Herbarium collection of the Center for Marine Research, Rovinj; 12 – Adam Mickiewicz University in Poznań. Natural History Collections of the Faculty of Biology AMU; 13 – Alexander J., Gross J. 2024. The University and Jepson Herbaria – Algae. Version 1.12. Berkeley Natural History Museums. A more detailed version is available as Table S1 in Supplementary Material. Notes: \*True collector was likely Hohenacker, year of collection is likely earlier; \*\*Misidentifications, for record purposes; \*\*\*Recently found misplaced specimen from Rovinj, marked as *Cystoseira* sp.; \*\*\*\*Illegible name, unknown location.

Name	Year	Location	Region, Country	Type	Source
de Wulfen	1803	Adriatic Sea	N/A	Uncertain	1
Naccari	1828	Trieste	Italy	Uncertain	2
Zanardini	1874	Adriatic Sea	N/A	Herbarium	3, 4
Keck/Hohenacker*	1878	Rovinj/Trieste	N/A	Herbarium	3
Hauck	1878	Rovinj	Istria, Croatia	Herbarium	4
Hansgirg	1885	Cres	Kvarner, Croatia	Herbarium	3
Zaratin	1885	Piran	Istria, Slovenia	Herbarium	5
Zaratin	1886	Butaceja islet, Funtana	Istria, Croatia	Herbarium	5
Zaratin	1886	Orada islet, Funtana	Istria, Croatia	Herbarium	5
Zaratin	1887	St. Katarina island, Rovinj	Istria, Croatia	Herbarium	5
Zaratin	1887	Rovinj	Istria, Croatia	Herbarium	5
Zaratin	1888	Pula	Istria, Croatia	Herbarium	5
Hansgirg**	1889	Rovinj	Istria, Croatia	Herbarium	3
Hansgirg**	1891	Old torpedo factory, Rijeka	Rijeka bay, Croatia	Herbarium	3
Hansgirg**	1891	Senj	Kvarner, Croatia	Herbarium	3
Hansgirg**	1891	Kaštel Novi	Split area, Croatia	Herbarium	3
Hansgirg**	1891	Split	Mid Adriatic, Croatia	Herbarium	3
Hansgirg**	1891	Gruž	Dubrovnik, Croatia	Herbarium	3
Lucas	1892	Rovinj	Istria, Croatia	Sampled	6
Kuckuck	1894	Banjole islet, Rovinj	Istria, Croatia	Sampled	6
Kuckuck	1895	Banjole islet, Rovinj	Istria, Croatia	Sampled	6
Schiller	1907	Novigrad	Istria, Croatia	Herbarium	3
Vatova	1914	Rovinj	Istria, Croatia	Observed	7
Ercegović	1965	Split area	Mid Adriatic, Croatia	Sampled	8
Ercegović	1965	Split area	Mid Adriatic, Croatia	Sampled	9
Špan	1965	Hvar island	Mid Adriatic, Croatia	Sampled	10
Zavodnik, N.***	1999	Banjole islet, Rovinj	Istria, Croatia	Herbarium	11
Hauck	N/A	Rovinj	Istria, Croatia	Herbarium	3
Meneghini****	N/A	Dalmatia	Croatia	Herbarium	4
Zanardini	N/A	Pula	Istria, Croatia	Herbarium	4
Zanardini	N/A	Pula	Istria, Croatia	Herbarium	4
Liechtenstern	N/A	Rovinj	Istria, Croatia	Herbarium	12
Radlkofi	N/A	Adriatic Sea	N/A	Herbarium	3, 13

in Istria, Shiller collected and preserved a specimen in Novigrad in 1907, which represents the last collected and preserved northern Adriatic specimen until recently (Table 1; Algae Herbarium Portal).

Among other written records, Vatova (1928) mentioned a washed-up specimen found in Valdibora – Rovinj in 1914, but the specimen was not preserved. Later, Vatova (1948), Ercegović (1966) and Giaccone (1978) all state in their publications that the species is present in the Adriatic, but without any details on its distribution, or simply citing old records. While Vatova (1928), Ercegović (1966, 1980) and later Špan (1980) based their statements on observed and/or collected specimens, the type of information provided by Giaccone (1978) is based on older literature and one unidentified herbarium specimen. Nonetheless, Giaccone's information is cited in Gómez Garreta *et al.* (2001), while Vatova and Ercegović are cited by Antolić *et al.* (2011) as a source of information for species' presence in the Adriatic. Ultimately, Guiry and Guiry (2024) state Gómez Garreta *et al.* (2001) and consequently Giaccone (1978), as the most up to date distribution data for the species covering the northern Adriatic. Unfortunately, it seems all the most recent checklists and databases (Guiry and Guiry, 2024; Antolić *et al.*, 2011, Gómez Garreta *et al.*, 2001) covering the northern Adriatic are citing the unconfirmed findings or, possibly indirectly, old herbarium collections and historical records from the late 19<sup>th</sup> and early 20<sup>th</sup> century, without providing any more recent records or newly collected specimens.

Temperatures in the northern Adriatic can fall below 11 °C during winter and approach 30 °C during summer (Bianchi, 2007). However, conditions in the very shallow coastal zone can vary significantly depending on the circulation, atmospheric conditions and geomorphology of the area. This can allow certain species, either thermophilic or cold-adapted, to exist despite the specific northern Adriatic conditions in the open waters (Iveša *et al.*, 2022). While considered a thermophilic, circum-tropical species (Orfanidis and Breeman, 1999; Guiry and Guiry, 2024), *D. simplex* could still persist in such areas, although to a lesser extent, which can contribute to its comparable scarcity in the northern Adriatic basin.

Historical records indicate that *D. simplex* was more commonly encountered in the northern Adriatic during 19<sup>th</sup> and early 20<sup>th</sup> century, since it was collected or noted by several scientists from Trieste in Italy (Battelli and Alberti, 2003), Piran in Slovenia to Pula in Croatia (Vatova, 1928; Munda, 2000; Battelli and Alberti, 2003; Algae Herbarium Portal). Despite nowadays being considered rare in the northern Adriatic, it is more likely that, due to its unremarkable appearance and occurrence in areas with heavy sedimentation, it was overlooked. On top of that, several historical specimens collected by Hansgirg in the late 1800s from Rovinj, Cres, Senj, Kaštel Novi, Split, and Gruž are misidentified (Algae Herbarium Portal; Table 1). This information was also compiled and intentionally included here for future records.

## CONCLUSIONS

*Digenea simplex* remains present in the northern Adriatic despite being missing from records and herbaria for a century, which is confirmed by the recently discovered washed-up specimens, misidentified herbarium specimens, and located extant settlements. Future plans include detailed surveys of historical areas and newly found settlements, genetic analyses and population comparison as well as an overview of species found in marine litter (targeting larger algae species) along the Istrian coastline.

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## AUTHOR CONTRIBUTIONS

Conceptualization, A.B., E.G. and L.J.I.; Methodology, A.B., E.G. and L.J.I.; Validation, A.B., E.G., L.J.I. and S.M.S.; Formal analysis, E.G.; Investigation, A.B., E.G. and L.J.I.; Resources, A.B., E.G. and L.J.I.; Data curation, E.G.; Writing – original draft, E.G.; Writing – review & editing, A.B., E.G., L.J.I. and S.M.S.; Visualization, E.G.; Supervision, L.J.I.; Project administration, L.J.I.; Funding acquisition, L.J.I.

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