

1 Title: Naturally occurring fluorescence protects the eutardigrade *Paramacrobiotus* sp.
2 from ultraviolet radiation

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4 Running title: Fluorescence and UV tolerance in tardigrades

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6 Authors: Harikumar R Suma¹, Swathi Prakash¹, Debasish Giri², Govindasamy
7 Mugesh², Sandeep M. Eswarappa^{1*}

8 ¹Department of Biochemistry, Indian Institute of Science, Bengaluru 560012,
9 Karnataka, India

10 ²Department of Inorganic and Physical Chemistry, Indian Institute of Science,
11 Bengaluru 560012, Karnataka, India

12

13 * Correspondence:

14 Sandeep M Eswarappa

15 Assistant Professor,

16 Department of Biochemistry,

17 Indian Institute of Science,

18 Bengaluru 560012,

19 Karnataka, India

20

21 Tel: 0091-80-22932881

22 Email: sandeep@iisc.ac.in

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27 **Keywords:** Tardigrade, Fluorescence, Ultraviolet radiation

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1 **Summary statement**

2 Tardigrades are well known for their tolerance to extreme environmental conditions.
3 In this study, we have identified a new tardigrade species that employs a fluorescent
4 shield to protect itself from the germicidal ultra violet radiation.

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7 **Abstract**

8 Naturally occurring fluorescence has been observed in multiple species ranging from
9 bacteria to birds. In macroscopic animals such as birds and fishes, fluorescence
10 provides a visual communication signal. However, the functional significance of this
11 phenomenon is not known in most cases. Though photoprotection is attributed to
12 fluorescence under ultraviolet (UV) light in some organisms, it lacks direct
13 experimental evidence. Here, we have identified a new species of eutardigrade
14 belonging to the genus *Paramacrobotus*, which exhibits fluorescence under UV light.
15 Using a natural variant of the same species that lacks fluorescence, we show that
16 the fluorescence confers tolerance to lethal UV radiation. Remarkably, we could
17 transfer this property to UV-sensitive *Hypsibius exemplaris*, another eutardigrade,
18 and also to *C. elegans*, a nematode. Using high performance liquid chromatography
19 (HPLC) we isolated the fluorescent compound from *Paramacrobotus* sp. This
20 compound has excitation maxima (λ_{ex}) at 370 nm and emission maxima (λ_{em}) at 420-
21 430 nm. We propose that *Paramacrobotus* sp. uses a fluorescent shield that
22 absorbs harmful UV radiation, and emits harmless blue light, thereby protecting itself
23 from the lethal effects of UV radiation.

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1 **Introduction**

2 Tardigrades (water bears) are microscopic (0.5 to 1 mm) limno-terrestrial animals
3 with four pairs of legs. More than a thousand species have been reported worldwide
4 from various habitats (Guidetti and Bertolani, 2005). *Hypsibius exemplaris*,
5 *Ramazzottius varieornatus*, *Richtersius coronifer*, *Milnesium tardigradum* and
6 *Paramacrobiotus richtersi* are some of the well-studied tardigrade species. Phylum
7 Tardigrada comes under the superphylum Ecdyzoa along with its sister phyla:
8 Arthropoda, Nematoda and Onychophora (Borner et al., 2014). An analysis based on
9 the *Hox* genes revealed that the entire tardigrade body is homologous to just the
10 head segment of arthropods (Smith et al., 2016).

11 Tardigrades are known for their tolerance to extreme environmental
12 conditions. Among them, their ability to tolerate complete desiccation is well known
13 (Goldstein and Blaxter, 2002). This is achieved by a reversible process called
14 anhydrobiosis where tardigrades shrink their size and slow-down their metabolism to
15 reach a physiological state called 'tun'. Tardigrades are also known to tolerate other
16 physical stresses including extreme temperature, pressure, ionizing radiations,
17 oxidative stress and osmotic variations (Altiero et al., 2011; Guidetti et al., 2011;
18 Hashimoto et al., 2016; Hengherr et al., 2009; Horikawa et al., 2013; Jonsson et al.,
19 2005; Ono et al., 2008; Rizzo et al., 2010; Tsujimoto et al., 2016). These harsh
20 conditions are lethal to most of the animals. Certain tardigrade species have even
21 survived the exposure to space vacuum and galactic radiations in low Earth orbit
22 conditions (Jonsson et al., 2008).

23 Unfortunately, the molecular and cellular mechanisms behind the
24 extraordinary stress tolerance of tardigrades are poorly understood. Of late, there is
25 an increase in the molecular studies that focus on the stress tolerance of tardigrades
26 (Harikumar and Eswarappa, 2017). A recent analysis of the genome of the
27 tardigrade *Ramazzottius varieornatus* revealed several potential mechanisms behind
28 its extreme radiotolerance. It lacks the genes that promote stress-induced damage
29 and there is a selective expansion of gene families responsible for decreasing
30 various stress-induced damages. This tardigrade has also evolved a unique protein
31 called damage suppressor protein (Dsup), which can impart partial radiotolerance to
32 cultured mammalian cells (Hashimoto et al., 2016). Another study showed that
33 tardigrade-specific intrinsically disordered proteins (TDPs) are required for
34 desiccation tolerance in *Hypsibius exemplaris* (Boothby et al., 2017). Expression of

1 these proteins is increased during desiccation and they form non-crystalline
2 amorphous solids. This vitrification process is implicated in the desiccation tolerance
3 of tardigrades.

4 Though fluorescence is abundant in marine organisms, it is not very common
5 in terrestrial animals. Fluorescence has been reported in parrots, scorpions,
6 chameleons, frogs, and nematodes (Arnold et al., 2002; Coburn et al., 2013; Lagorio
7 et al., 2015; Protzel et al., 2018; Taboada et al., 2017). Functional significance of this
8 phenomenon is unclear although visual signalling towards potential mates has been
9 attributed in case of parrots (Arnold et al., 2002). Fluorescence has been reported in
10 tardigrades also, but its function is unknown (Perry et al., 2015).

11 Here we show that an eutardigrade *Paramacrobiotus* sp. exhibits tolerance
12 to germicidal ultraviolet (UV) radiation up to one hour. These tardigrades also show
13 fluorescence under UV light. We demonstrate that this phenomenon contributes to
14 their exceptional tolerance to UV radiation.

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1 **Materials and methods**

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3 **Tardigrade culture**

4 *Paramacrobiotus* sp. was isolated from moss samples grown on a concrete wall
5 inside Indian Institute of Science campus (13° 0' 55" N and 77° 33' 57" E),
6 Bangalore, India. The concrete wall was well lit by direct sunlight most of the days in
7 a year. Moss samples were kept in a 90 mm petri dish immersed in reverse osmosis
8 (RO) water for 12 h. Samples were gently tapped to facilitate the dislodgement of
9 tardigrades to the water in the petri dish, which were then visualized under an upright
10 light microscope (CXL Plus, LABOMED, USA). Once spotted, tardigrades were
11 taken out using a pipette, rinsed in RO water and placed in an embryo culture dish.
12 They were cultured in KCM solution (7 mg KCl, 8 mg CaCl₂, and 8 mg MgSO₄·7H₂O
13 in 1 L of water) in 35 mm petri dishes coated with 2% low EEO agarose (Lonza,
14 Switzerland) at 20° C (Suzuki, 2003). Cultures were kept in dark and *Caenorhabditis*
15 *elegans* and rotifers were provided as food source. *Hypsibius exemplaris* (previously
16 known as *Hypsibius dujardini* Z151) was a kind gift from Prof. Bob Goldstein
17 University of North Carolina at Chapel Hill, USA. They were cultured as described
18 previously in Chalkley's medium with algae (*Chlorococcum* sp.) as food source
19 (Gabriel et al., 2007). Prior to experiments, tardigrades were washed thoroughly
20 using RO water and starved for 24 h in RO water containing Ampicillin (0.5 mg/ml).
21 *C. elegans* (Bristol N2 strain) was a kind gift from Dr. Varsha Singh, Indian Institute
22 of Science, Bengaluru. Worms were maintained in Nematode Growth Medium
23 (NGM) with *E. coli* OP50, as the food source.

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25 **Identification of tardigrades by sequencing**

26 Genomic DNA was extracted from a single individual of the newly isolated tardigrade
27 by using a rapid salt and ethanol precipitation as described before (Cesari et al.,
28 2009). A single tardigrade was homogenised in 100 µl of TNES extraction buffer (50
29 mM Tris pH 7.8, 400 mM NaCl, 20 mM EDTA, 0.5% SDS) followed by proteinase K
30 (0.01 µg/µl) treatment for 2 h at 55° C. To precipitate proteins, 1M NaCl was added
31 and the mixture was centrifuged at 18,000 g for 5 min and the supernatant was
32 collected. Equal volumes of phenol-chloroform-isoamyl alcohol was added to the
33 supernatant and centrifuged at 20,000 g for 5 min. Genomic DNA was precipitated
34 using 70% ethanol and 0.3 M sodium acetate and resuspended in 20 µl of nuclease-

1 free water. PCR reactions were carried out using 200 ng of genomic DNA and
2 universal primers for *COI* (Mitochondrial cytochrome oxidase 1) gene and ITS2
3 (nuclear Internal Transcribed spacer 2) region as described previously (Folmer et al.,
4 1994; White et al., 1990). The PCR product was subjected to Sanger sequencing.
5 Obtained sequence was subjected to BLAST analysis to identify the closest
6 tardigrade species.

7 Primers for *COI*: 5'-GGTCAACAAATCATAAAGATATTGG-3'
8 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

9 Primers for *ITS2*: 5'-GCATCGATGAAGAACGCAGC-3'
10 5'-TCCTCCGCTTATTGATATGC-3'

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12 **Phylogenetic analysis**

13 The *COI* gene (encodes Cytochrome C oxidase subunit I) sequences of various
14 tardigrade species belonging to the following families were retrieved from NCBI
15 (species name and accession number are given in parenthesis): Macrobiotidae
16 (*Paramacrobotus richtersi* group 2 from Kenya [EU244598], *Paramacrobotus*
17 *richtersi* group 3 from Kenya [EU244599], *Paramacrobotus richtersi* isolate
18 CLARE_ISLAND-3 [MK040994], *Paramacrobotus richtersi* isolate CLARE_ISLAND-
19 2 [MK040993], *Macrobiotus pallarii* isolate Tar407 [FJ435807], *Paramacrobotus*
20 *lachowskiae* [MF568534], *Macrobiotus papei* [MH057763], *Macrobiotus macrocalix*
21 isolate PL.110 [MH057767], *Macrobiotus polypiformis* haplotype 1 [KX810011],
22 *Macrobiotus hannaе* strain PL.010 [MH057764], *Macrobiotus shonaicus* haplotype 1
23 [MG757136]), Hypsibiidae (*Hypsibius exemplaris* strain Sciento Z151 [MG818724],
24 *Ramazzottius varieornatus* [MG432813], *Hypsibius klebelsbergi* voucher HD070
25 [KT901832]), Milnesiidae (*Milnesium tardigradum* from Japan [EU244604],
26 *Milnesium variefidum* [KT951663], *Milnesium berladicorum* [KT951659]), and
27 Echiniscidae (*Echiniscus testudo* from Egypt [EU244601], *Echiniscus cf. testudo* Et-
28 Ladakh-1 [EF620367], *Echiniscus blumi* haplotype 1 [EU046090]). The *COI*
29 sequence of newly identified tardigrade species was obtained by sanger sequencing
30 as described above.

31 Phylogenetic tree was constructed using MEGA X software (ver. 10.1.5). The
32 *COI* sequences were aligned using ClustalW option in MEGA X software. All the
33 sequences were trimmed to make them of uniform length. Maximum Likelihood

1 analysis was performed with 1000 bootstrap replicates using a General Time
2 Reversible (GTR) + G+I model.

3

4 **Microscopy**

5 Tardigrades and their eggs were placed on a glass slide with two drops of saline
6 (0.9%) solution. Wet mounts were prepared by gently placing a coverslip over the
7 saline drop without damaging the tardigrades. Excess fluid was removed using a lint-
8 free tissue paper. Bright field images were captured using Axio Scope upright light
9 microscope (Carl Zeiss, Germany). DIC (Differential interference contrast) and
10 fluorescence images were obtained using Axio Observer.Z1 inverted fluorescence
11 microscope (Carl Zeiss, Germany) equipped with an HBO 100 lamp. Band pass
12 filters were used for excitation and emission (G365 and BP 445/50 (Carl Zeiss)).
13 DAPI channel was used to capture fluorescence images with exposure time constant
14 at 1 s during the image acquisition. The acquired images were analyzed using Axio
15 Vision imaging software (version: 4.8.2.0; Carl Zeiss, Germany).

16

17 **UV Irradiation**

18 Three groups of ten individuals from both tardigrade species were taken in 35 mm
19 petri dishes coated with 2% low EEO agarose and excess water was removed. The
20 animals remained hydrated for the duration of experiment as they were in contact
21 with the moist agarose surface (Horikawa et al., 2013). They were immediately
22 exposed to UV radiation (peak wavelength 253 nm) emanating from a germicidal
23 lamp (LT-T8 30W/UV-C HRA, Narva, Germany). The irradiance of the beam was
24 0.111 mW/cm^2 as measured by an UV radiation meter (UVITEC, UK). The UV dose
25 was calculated according to the formula $1 \text{ (mW/cm}^2\text{)sec} = 1 \text{ mJ/cm}^2$ as described
26 previously (Horikawa et al., 2013). Tardigrades were exposed to multiple doses (0.6
27 kJ/m^2 , 1 kJ/m^2 , 2 kJ/m^2 , 4 kJ/m^2 and 8 kJ/m^2) of UV light by varying the duration of
28 exposure. After the exposure, samples were transferred to fresh 2% agarose-coated
29 35 mm petri dishes and cultured as described above. They were monitored daily
30 under a light microscope for a period of 30 days. Any eggs laid were collected and
31 cultured separately to check their hatchability.

32 To test if the UV resistant property of *Paramacrobotus* sp. can be transferred
33 to UV sensitive *H. exemplaris* or *C. elegans*, three hundred individuals of
34 *Paramacrobotus* sp. were homogenized in 120 μl of water using a mechanical tissue

1 grinder. This was followed by centrifugation of the lysate at 20,000 g for 15 min. The
2 supernatant showed fluorescence under UV light (wavelength 254 nm and 365 nm)
3 and 40 μ l of it was added in a well of a 96-well plate. 40 μ l of sterile water was used
4 as control. Twenty individuals of *H. exemplaris* or 50 individuals of *C. elegans* were
5 added in those wells, and exposed to UV-C radiation (1 kJ/m²) as described above.
6 They were monitored every day after the treatment.

7

8 **Methanol extraction of tardigrades**

9 Five hundred individuals of *Paramacrobotus* sp. were transferred into a 1.5 ml tube
10 containing 200 μ l methanol. The tube was subjected to a freeze-thaw cycle in liquid
11 nitrogen followed by mechanical homogenization using a tissue grinder. The lysate
12 was centrifuged at 20,000 g for 5 min and the supernatant was collected. This
13 process was repeated twice and the supernatants were pooled. As a control, 1000
14 individuals of *H. exemplaris* were subjected to the same extraction procedure and
15 the supernatant was collected. The extracts were then visualized under a UV lamp
16 (GeNeiTM, India) to observe the fluorescence (excitation wavelengths were: 254 nm
17 and 365 nm).

18

19 **High Performance Liquid Chromatography (HPLC)**

20 100 μ l of methanolic extract of tardigrades was injected into Waters HPLC system
21 with a reverse phase column (Hibar[®] RT C-18 column; 4.6x250 mm, particle size: 5
22 μ m) using an auto sampler. A gradient of acetonitrile/water was used as the mobile
23 phase at a flow rate of 1 ml/min. A PDA detector was used to detect absorbance at
24 350 nm. The fraction corresponding to the unique peak around 5.5 to 6 min in
25 *Paramacrobotus* sp. extract was collected from the flow-through. After confirming
26 the fluorescence of this fraction, it was lyophilized and re-suspended in methanol.
27 Fluorescence properties were investigated using a spectrofluorometer (FP-6300,
28 Jasco, USA). As a control the HPLC fraction of *H. exemplaris* was used. Excitation
29 profile was obtained by varying the emission wavelength between 400 nm and 460
30 nm. Emission profile was obtained by varying the excitation wavelength between 300
31 nm and 370 nm. The spectral profiles were corrected by subtracting the values of
32 methanol.

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1 Results

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3 **The new tardigrade isolate belongs to the genus *Paramacrobotus***

4 We identified a new species of tardigrade from moss samples growing on a concrete
5 wall at Indian Institute of Science, India (13° 0' 55" N and 77° 33' 57" E; see Methods
6 for details). To identify the species, we sequenced the *COI* (Mitochondrial
7 cytochrome oxidase 1) gene and *ITS-2* (nuclear Internal Transcribed spacer 2)
8 region from a single individual using universal primers as described previously
9 (Folmer et al., 1994; White et al., 1990). BLAST analysis of the obtained sequences
10 against all available nucleotide sequences in NCBI revealed their high similarity to
11 the sequences of *COI* (81.62% identity) and *ITS-2* (85.96% identity) from the
12 eutardigrade *Paramacrobotus richtersi*. Phylogenetic analysis based on these
13 sequences revealed that the newly identified tardigrades belong to the genus
14 *Paramacrobotus* under the family Macrobiotidae, class Eutardigarda and phylum
15 Tardigrada (Fig 1A and Table S1, S2). Henceforth the new tardigrade species will be
16 referred to as *Paramacrobotus* sp.

17 These tardigrades were about 600 µm long and had reddish-brown
18 pigmentation (Fig 1B). Close examination of the buccopharyngeal (feeding)
19 apparatus of the new isolate showed a cylindrical buccal tube (mouth tube) attached
20 to an oval shaped pharynx. The mouth opening was surrounded by buccal lamellae
21 in a circular arrangement mounted on the buccal crown. The feeding apparatus had
22 anterior macropiloids and posterior micropiloids with piercing stylets in a bent
23 position (Fig 1C). Hind legs had two Y-shaped double claws (Fig 1D). The eggs laid
24 by this tardigrade were spherical and ornamented with conical surface projections.
25 They were deposited outside the moulted cuticle (Fig 1E). All these morphological
26 features are characteristic of the family Macrobiotidae to which the genus
27 *Paramacrobotus* belongs (Guidetti et al., 2012; Schill, 2018).

28

29 ***Paramacrobotus* sp. shows tolerance to UV radiation**

30 Since tardigrades are known for their tolerance to extreme conditions, we exposed
31 the *Paramacrobotus* sp. to multiple physical stresses. We observed that these
32 tardigrades were particularly resistant to UV radiation. All of the *Paramacrobotus* sp.
33 survived 10 min exposure (corresponds to 0.66 kJ/m²) to germicidal UV radiation,
34 whereas all of the *Hypsibius exemplaris*, another eutardigrade species, died within

1 minutes after the same treatment (Fig 2A). Furthermore, 60% of *Paramacrobilotus*
2 sp. survived 1 h exposure to UV radiation (corresponds to 4 kJ/m²) beyond 30 days
3 (Fig 2B, C and D). The survived tardigrades were observed daily; they laid eggs that
4 hatched to normal individuals. This was observed for two generations showing that
5 UV exposure did not affect their survival or their reproductive ability.

6

7 **Naturally occurring fluorescence of *Paramacrobilotus* sp. is essential for its** 8 **tolerance to UV radiation**

9 Incidentally, *Paramacrobilotus* sp. showed strong fluorescence under UV illumination
10 (DAPI channel, Axio Observer.Z1). This fluorescence was absent in *H. exemplaris*,
11 which were sensitive to UV radiation. Similar fluorescence was observed in the eggs
12 of *Paramacrobilotus* sp., but not on its moulted cuticle (Fig 3A). Extract from
13 *Paramacrobilotus* sp. obtained after homogenizing the organisms in tissue lysis
14 buffer also showed strong fluorescence under UV illumination (254 nm and 365 nm).
15 The fluorescence was absent in the extract from *H. exemplaris*. The fluorescence
16 was intact even after proteinase K treatment of the lysate for one hour suggesting
17 that the fluorescent compound is not a protein (Fig 3B).

18 Occasionally we come across hypopigmented *Paramacrobilotus* sp. during
19 isolation. Morphological features and the nucleotide sequence of *ITS2* region
20 revealed that the hypopigmented and the pigmented tardigrades belong to the same
21 species (Figure 4A and S1). Interestingly, hypopigmented *Paramacrobilotus* sp.
22 showed much less fluorescence under UV light (Figure 4A and B). When they were
23 exposed to UV radiation for one hour, hypopigmented tardigrades showed
24 significantly less UV tolerance compared to the pigmented ones. All hypopigmented
25 *Paramacrobilotus* sp. died within 20 days after UV exposure, whereas 60% of the
26 pigmented *Paramacrobilotus* sp. survived beyond 30 days (Fig 4C). This observation
27 suggests that the newly identified *Paramacrobilotus* sp. uses fluorescence as a
28 mechanism to resist harmful UV radiation.

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30 **UV tolerance property of *Paramacrobilotus* sp. can be transferred to UV** 31 **sensitive *H. exemplaris* and *C. elegans*.**

32 We tested if the UV tolerance can be transferred to UV sensitive *H. exemplaris*.
33 For this, we homogenized 300 *Paramacrobilotus* sp. tardigrades in water. The
34 supernatant of the homogenate was fluorescent under UV light (Fig 5A). UV

1 sensitive *H. exemplaris* were covered in this fluorescent extract and exposed to UV
2 radiation for 15 min (corresponds to 1 kJ/m²). *H. exemplaris* covered in water were
3 used as control (Fig 5B). Interestingly, *H. exemplaris* tardigrades covered in the
4 fluorescent extract showed partial tolerance to UV radiation. To further confirm the
5 role of fluorescence, we photobleached the aqueous extract of *Paramacrobotus* sp.
6 by exposing it to 254 nm UV light. Bleached sample that lacked fluorescence was
7 used for the experiment described above. Unlike the fluorescent extract of
8 *Paramacrobotus* sp., the photobleached non-fluorescent extract from the same did
9 not confer UV tolerance on *H. exemplaris*. Similarly, extracts from hypopigmented
10 *Paramacrobotus* sp., which showed much reduced fluorescence, also could not
11 confer UV tolerance on *H. exemplaris* (Fig 5C and D). Remarkably, the fluorescent
12 extract of *Paramacrobotus* sp. could confer partial UV resistance on *C. elegans*, a
13 nematode (Fig 5E). Together, these results demonstrate that the fluorescence of
14 *Paramacrobotus* sp. is responsible for its UV tolerance.

15

16 **Properties of the fluorescent compound from *Paramacrobotus* sp.**

17 We used methanol to extract the fluorescent compound from the newly identified
18 tardigrade species. The methanolic extract from *Paramacrobotus* sp. was
19 fluorescent under UV light, whereas the extract from *H. exemplaris* was not (Fig 6A).
20 We then subjected the methanolic extract of *Paramacrobotus* sp. to High
21 Performance Liquid Chromatography (HPLC) to isolate the fluorescent compound.
22 We observed a unique peak (absorbance at 350 nm) in the extract from
23 *Paramacrobotus* sp. near 6 min, which was absent in the extract from UV-sensitive
24 *H. exemplaris* (Fig 6B). The sample collected from this unique fraction of
25 *Paramacrobotus* sp. exhibited fluorescence under UV light confirming the isolation
26 of fluorescent compound (Fig 6C). As expected, the fluorescent peak between 5 and
27 6 min in the HPLC profile of hypopigmented *Paramacrobotus* sp. was much smaller
28 compared to that in HPLC profile of pigmented *Paramacrobotus* sp. (Fig 6D).
29 Analysis using a spectrofluorometer showed that this fluorescent compound has
30 excitation maxima (λ_{ex}) at 370 nm and emission maxima (λ_{em}) at 420-430 nm.
31 Fluorescence was observed in a broad range of the UV spectrum between 250 to
32 370 nm (Fig 7A-C).

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1 Discussion

2 Fluorescence has been observed in diverse life forms - plants, worms, insects, frogs,
3 birds, etc. (Lagorio et al., 2015). Functional significance of this phenomenon is
4 known only in few organisms. Communication (e.g., mate attraction in zebra finch
5 birds and luring preys in siphonophores) seems to be one of the primary
6 physiological functions of fluorescence. However, the purpose of this phenomenon is
7 not clear in most cases. Photoprotective role has been suggested for fluorescence in
8 some organisms such as amphioxus, comb jellies and corals. In case of corals, a
9 strong correlation has been demonstrated between fluorescence and the
10 susceptibility to photo-inhibition and bleaching (Salih et al., 2000). However, there is
11 no direct experimental demonstration of photoprotection imparted by fluorescence in
12 any organism.

13 In this study, we show that a newly identified tardigrade species belonging to
14 the genus *Paramacrobotus* uses fluorescence to protect itself from UV radiation-
15 induced death. A non-fluorescent variant of the same species was susceptible to UV
16 radiation. Remarkably, we could transfer this property to a UV-sensitive tardigrade,
17 *H. exemplaris*, and to *C. elegans*. Addition of fluorescent aqueous extract of
18 *Paramacrobotus* sp. on the surface of UV-sensitive *H. exemplaris* made it partially
19 resistant to UV radiation. This was not observed when we used photobleached non-
20 fluorescent extract or extract from hypopigmented *Paramacrobotus* sp. These
21 experiments demonstrate that fluorescence is responsible for UV tolerance in
22 *Paramacrobotus* sp. Thus, fluorescent pigment serves as a shield against UV
23 radiation protecting these tardigrades from its lethal effects (Fig S2).
24 *Paramacrobotus* sp. has probably evolved this mechanism to counter high UV
25 radiation of tropical southern India from where it was isolated. It is possible that
26 these tardigrades have other mechanisms to protect themselves from UV radiation-
27 induced damage. For example, robust DNA repair pathways. Analysis of its genome
28 sequence will provide more insights.

29 Lethal effects of UV radiation are primarily due to DNA damage. It results in
30 cyclobutane-pyrimidine dimers (CPDs) and 6-4 photoproducts (pyrimidine adducts)
31 in genomic DNA, which affects replication and transcription. They can also cause
32 lethal mutagenic effects (Sinha and Hader, 2002). UV radiation also damages DNA
33 indirectly by producing reactive oxygen species. There are several organisms that
34 resist lethal effects of UV radiation using multiple mechanisms. *Deinococcus*

1 *radiodurans* has developed an efficient DNA repair pathway, which is responsible for
2 its resistance to high ionizing radiation as well as UV radiation (Krisiko and Radman,
3 2013). Production of pigments/compounds that absorb UV radiation is another
4 mechanism commonly found in organisms from bacteria to mammals (Singh and
5 Gabani, 2011). Cyanobacteria and other microbes produce UV-absorbing
6 compounds such as scytonemin, myosporine and related amino acids (Oren and
7 Gunde-Cimerman, 2007; Rastogi and Incharoensakdi, 2014). *Halobacterium*
8 *salinarium*, a red pigmented bacterium, produces bacterioruberin which protects it
9 from UV radiation (Dummer et al., 2011). Melanin in mammals and hipposudoric acid
10 (red sweat) in hippopotamus are other examples of pigments that absorb UV
11 radiation (Saikawa et al., 2004; Slominski et al., 2004). *Sander vitreus* (walleye) is a
12 golden yellow fish found in North American lakes. Increased incidences of blue
13 coloured walleye in recent years is thought to be an adaptation to increased UV
14 radiation. Sandercyanin–Biliverdin complex is responsible for this blue colour. This
15 complex exhibits red fluorescence under UV light (λ_{ex} 375 nm / λ_{em} 675 nm). However,
16 it is not known whether this complex and its fluorescence protect the fishes from
17 harmful effects of UV radiation (Ghosh et al., 2016). Our study adds fluorescence in
18 the tardigrade *Paramacrobiotus* sp. to the list of UV-protection mechanisms. The
19 chemical composition of this fluorescent compound remains to be investigated.

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5 **Competing Interests**

6 None of the authors have any competing interests.

7

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1 **Figure Legends**

2 **Figure 1. Newly isolated tardigrade species belongs to the genus**
3 ***Paramacrobotus*.** (A) Maximum likelihood phylogenetic tree based on *COI*
4 sequence of various tardigrades. The tree was constructed using MEGA X software.
5 Newly identified species is shown in bold. *COI* sequences of other tardigrade
6 species were taken from NCBI. Numbers show bootstrap values. (B-E)
7 Morphological features of the new species. The entire body with reddish-brown
8 pigmentation (B), buccopharyngeal (feeding) apparatus (C), hind legs with two Y-
9 shaped double claws (D), and ornamented eggs with surface projections (E) are
10 shown.

11

12 **Figure 2. *Paramacrobotus* sp. shows tolerance to UV radiation.** Survival of
13 *Paramacrobotus* sp. under UV radiation exposure for 10 min (0.66 kJ/m²) (A), for 30
14 min (2 kJ/m²) (B), and for 1 hour (4 kJ/m²) (C) is shown. *H. exemplaris*, another
15 eutardigrade species, was used as control. Comparison of survival of
16 *Paramacrobotus* sp. to different doses of UV radiation is shown in (D). Each point in
17 the graph shows mean \pm SE, n=10x3. Statistical significance was calculated using
18 log-rank test.

19

20 **Figure 3. *Paramacrobotus* sp. shows fluorescence under UV light.** (A)
21 Fluorescence microscope images of *Paramacrobotus* sp., its moulted cuticle and
22 egg are shown. UV-sensitive *H. exemplaris* was used as control for comparison. All
23 images were taken under identical microscope settings. (B) Fluorescence images of
24 the total lysates of *Paramacrobotus* sp. and *H. exemplaris* under UV light (365 nm
25 and 254 nm) are shown. *Paramacrobotus* sp. lysate shows fluorescence that is
26 stable even after proteinase K treatment.

27

28 **Figure 4. Hypopigmented *Paramacrobotus* sp. does not show fluorescence**
29 **and does not exhibit UV tolerance.** (A) Images of hypopigmented
30 *Paramacrobotus* sp. showing reduced fluorescence under UV light compared to
31 pigmented ones. (B) Graph showing the quantification of fluorescence. Bars show
32 mean \pm SE, n=6. Statistical significance was calculated using two-tailed Student's t-
33 test. (C) Survival of hypopigmented *Paramacrobotus* sp. under UV radiation

1 exposure for 1 hour (4 kJ/m^2). Each point in the graph shows mean \pm SE, $n=10 \times 3$.
2 Statistical significance was calculated using log-rank test.

3

4 **Figure 5. Transfer of UV tolerance property from *Paramacrobotus* sp. to *H.***
5 ***exemplaris* and *C. elegans*.** (A) Fluorescence of aqueous extract from
6 *Paramacrobotus* sp. under UV light. (B) Schematic of the experimental setup. (C)
7 Fluorescence of aqueous extract from *Paramacrobotus* sp., photobleached extract
8 and extract from hypopigmented strain. (D) Survival of *H. exemplaris* tardigrades
9 incubated with extracts shown in (C) under UV radiation. (E) Survival of *C. elegans*
10 incubated with fluorescent aqueous extract from *Paramacrobotus* sp. under UV
11 radiation. Each point in the graphs shown in (D) and (E) represents mean \pm SE,
12 $n=20 \times 3$ (in D) or $n=50 \times 3$ (in E). Statistical significance was calculated using log-rank
13 test. Para, *Paramacrobotus* sp.

14

15 **Figure 6. Isolation of fluorescent compound from *Paramacrobotus* sp. by**
16 **HPLC.** (A) UV fluorescence of methanolic extracts from *Paramacrobotus* sp. (B)
17 HPLC profiles (absorbance at 350 nm) of methanolic extracts from *Paramacrobotus*
18 sp. and *H. exemplaris*. Arrow indicates the peak unique to *Paramacrobotus* sp. UV
19 fluorescence of the fraction from this peak is shown in (C). (D) HPLC profiles
20 (absorbance at 350 nm) of methanolic extracts from hypopigmented
21 *Paramacrobotus* sp.

22

23 **Figure 7. Spectral properties of fluorescent compound from *Paramacrobotus***
24 **sp.** Fluorescent HPLC fraction shown in Fig 6C was lyophilized and resuspended in
25 methanol. Spectral property was analysed using spectrofluorometer. (A) Excitation
26 scan: fluorescence intensity of the compound when excited using UV light of
27 wavelengths ranging from 300 nm to 370 nm. (B) Relatively weaker fluorescence
28 was seen at UV radiation of lower wavelength (250 nm, 254 nm and 260 nm). Two-
29 times more concentrated extract was used for the analysis shown in (B) compared to
30 the same in (A). (C) Emission scan: fluorescence intensity of the compound at
31 multiple emission wavelengths (400 nm to 460 nm).

Figure 1

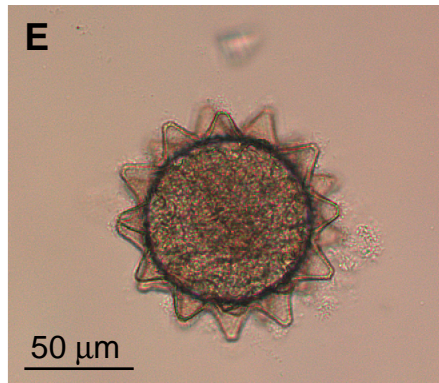
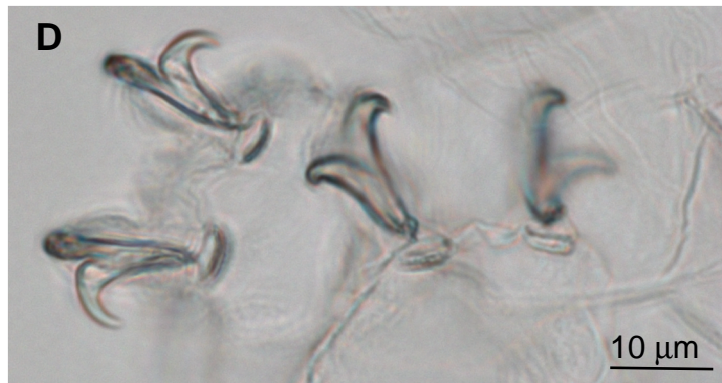
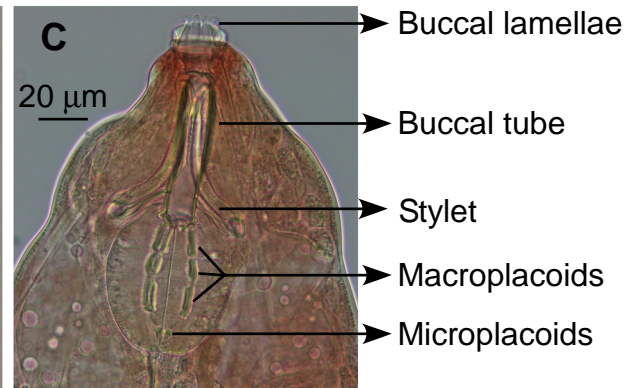
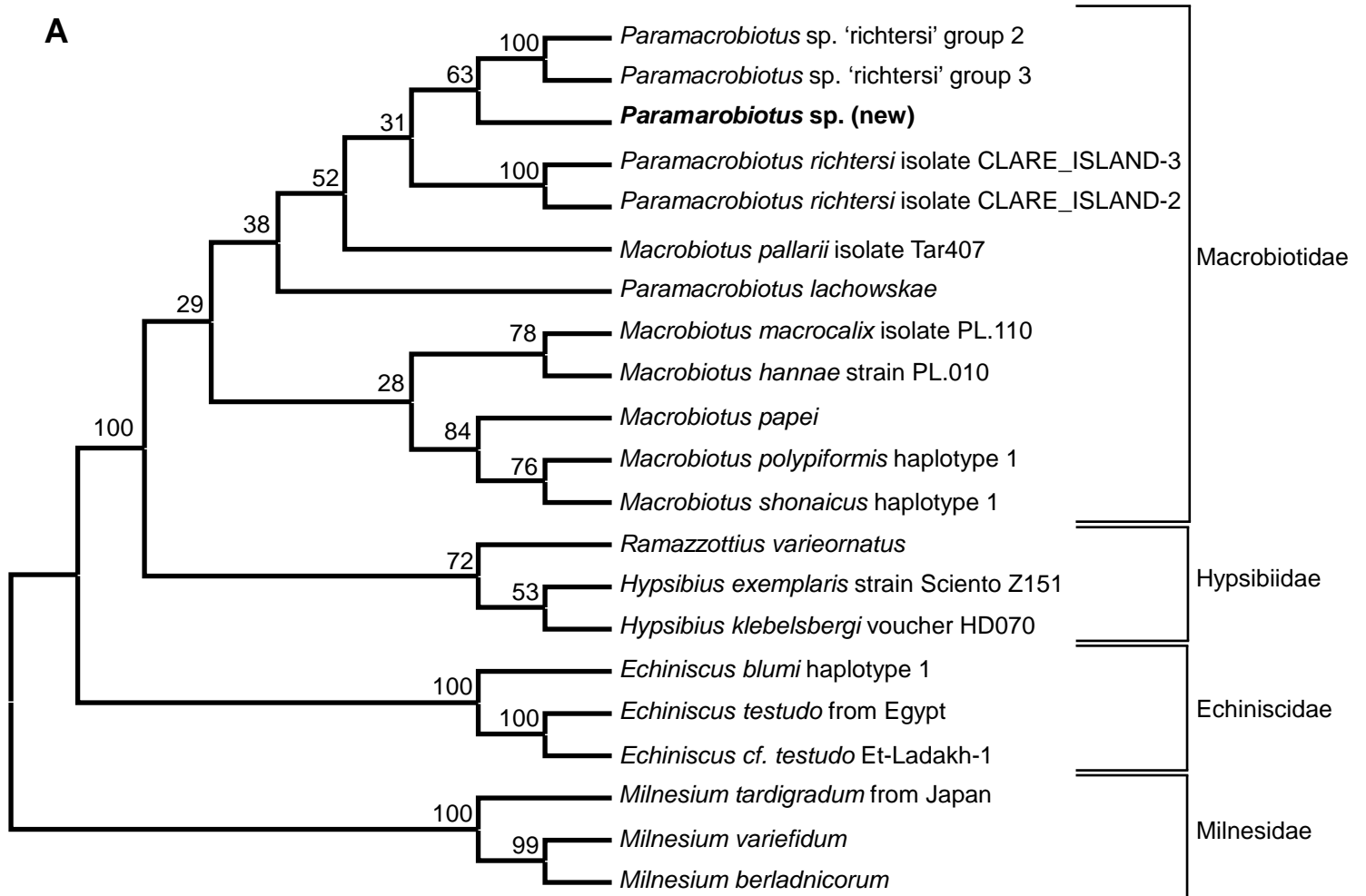


Figure 2

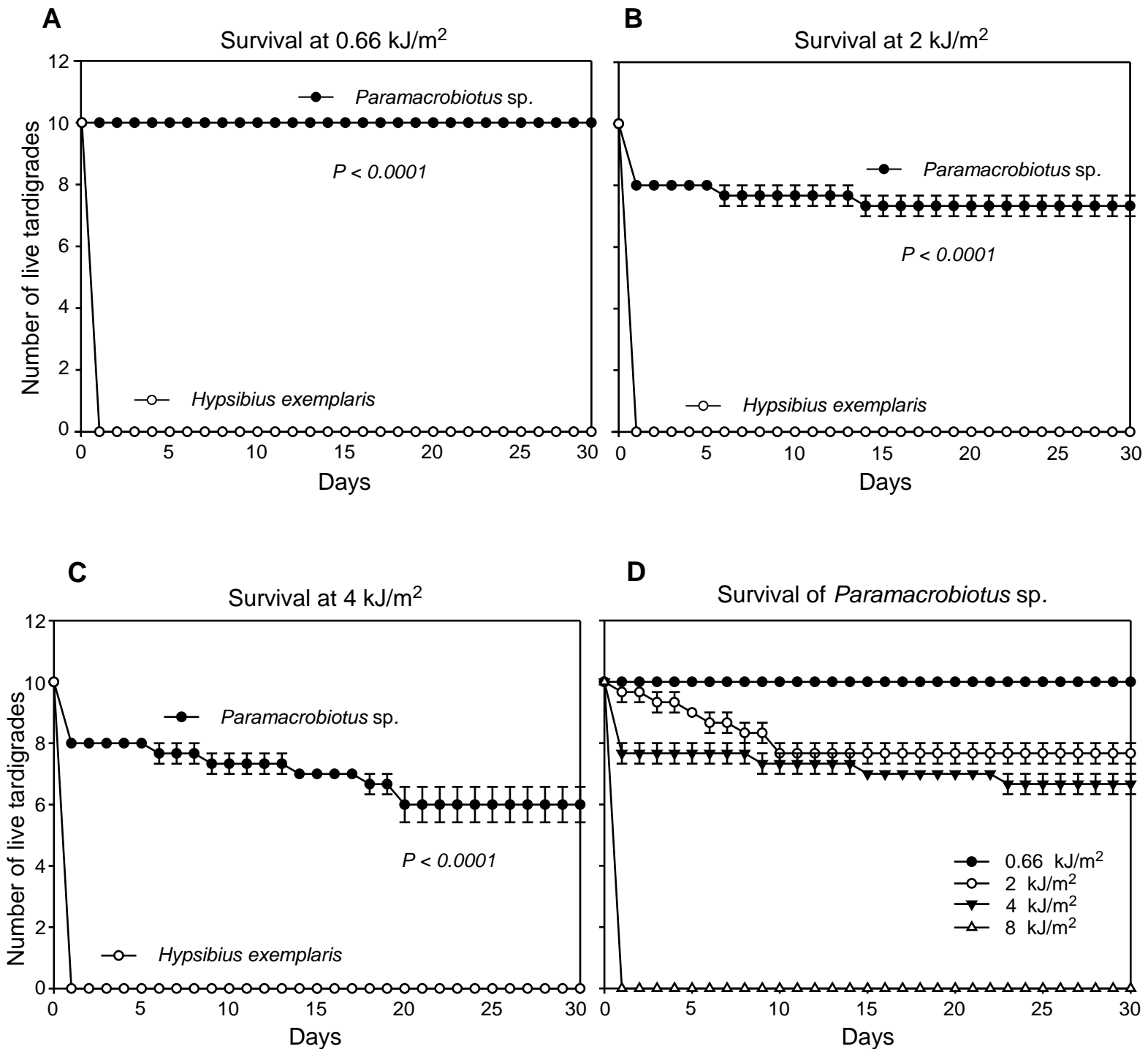


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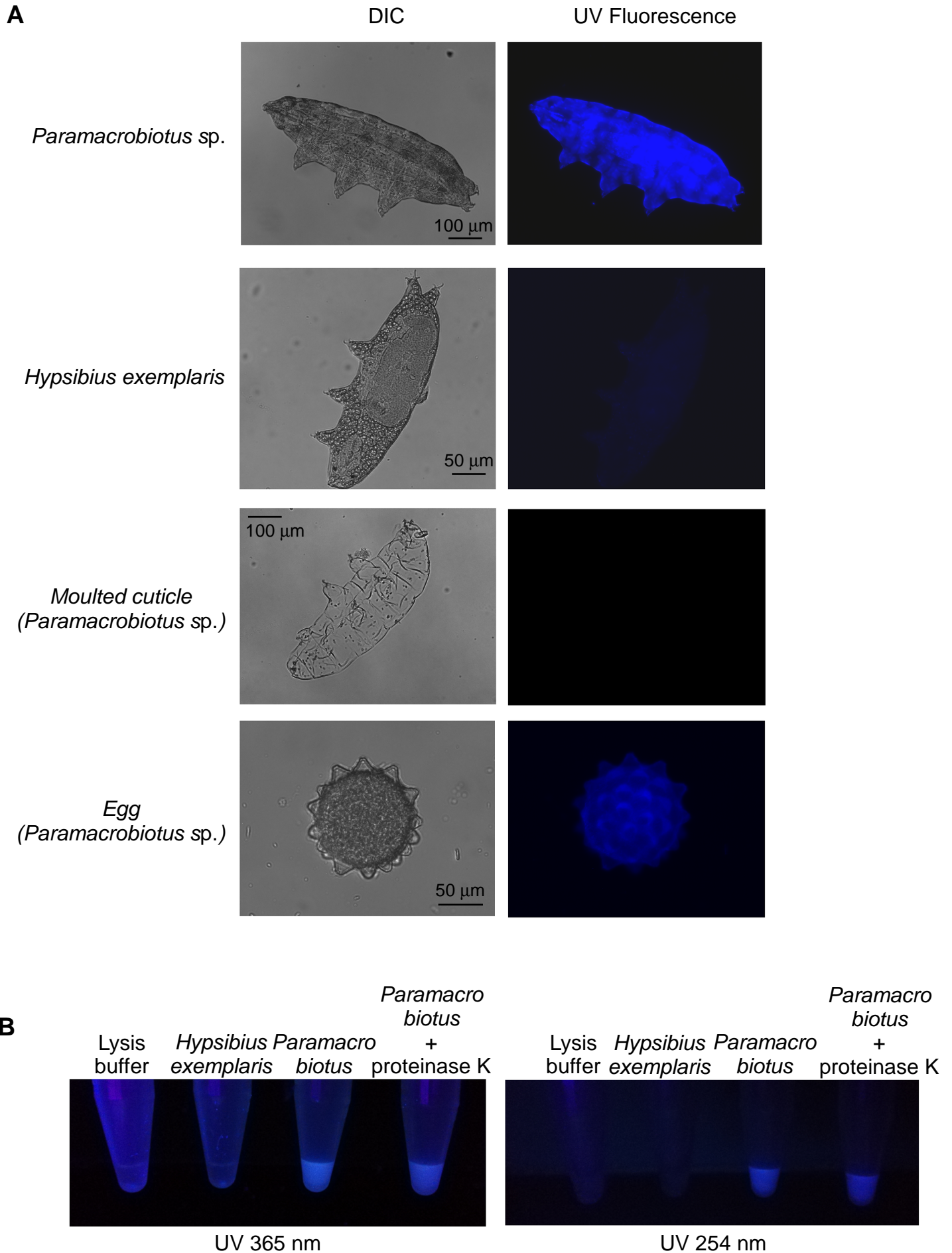


Figure 4

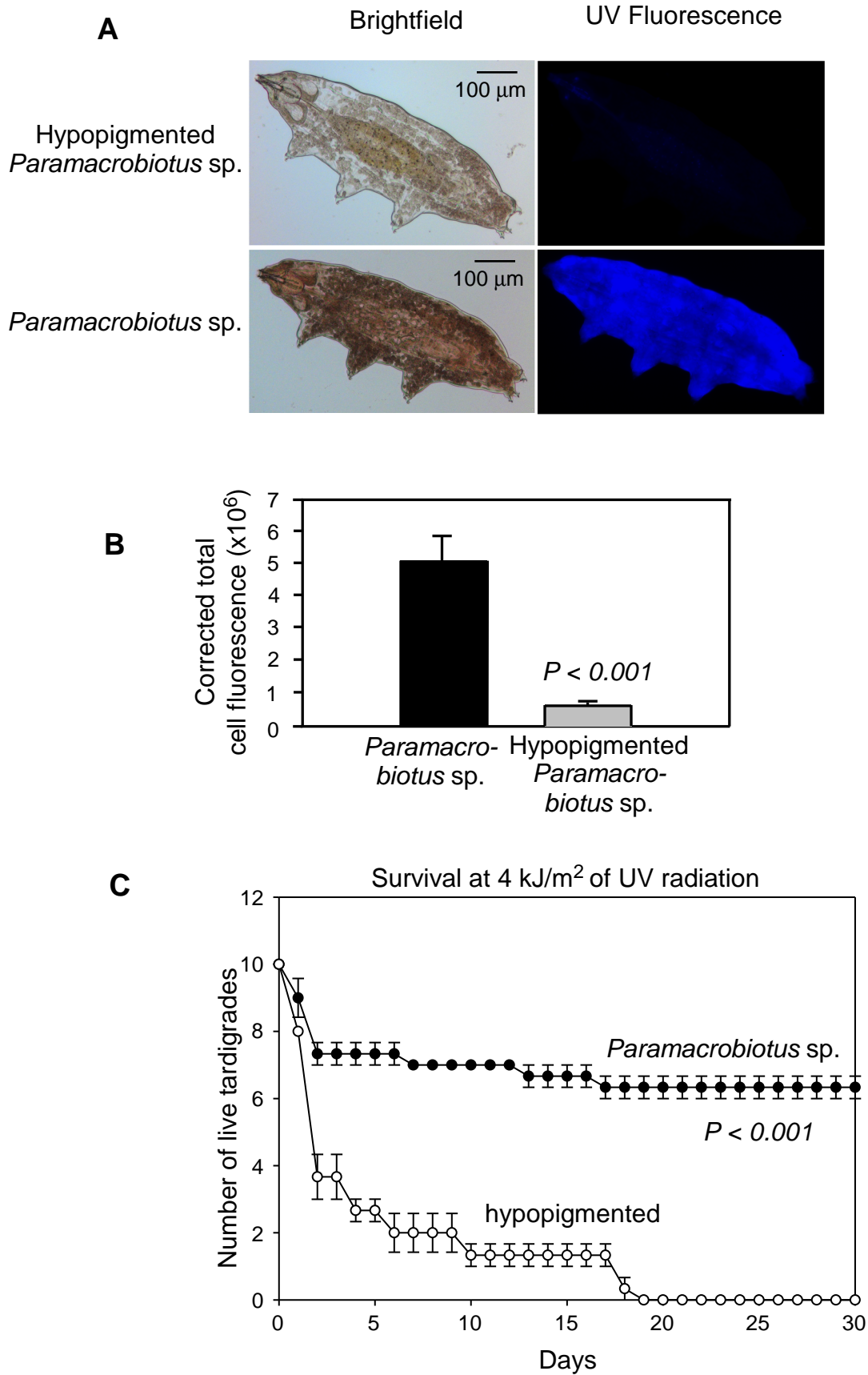
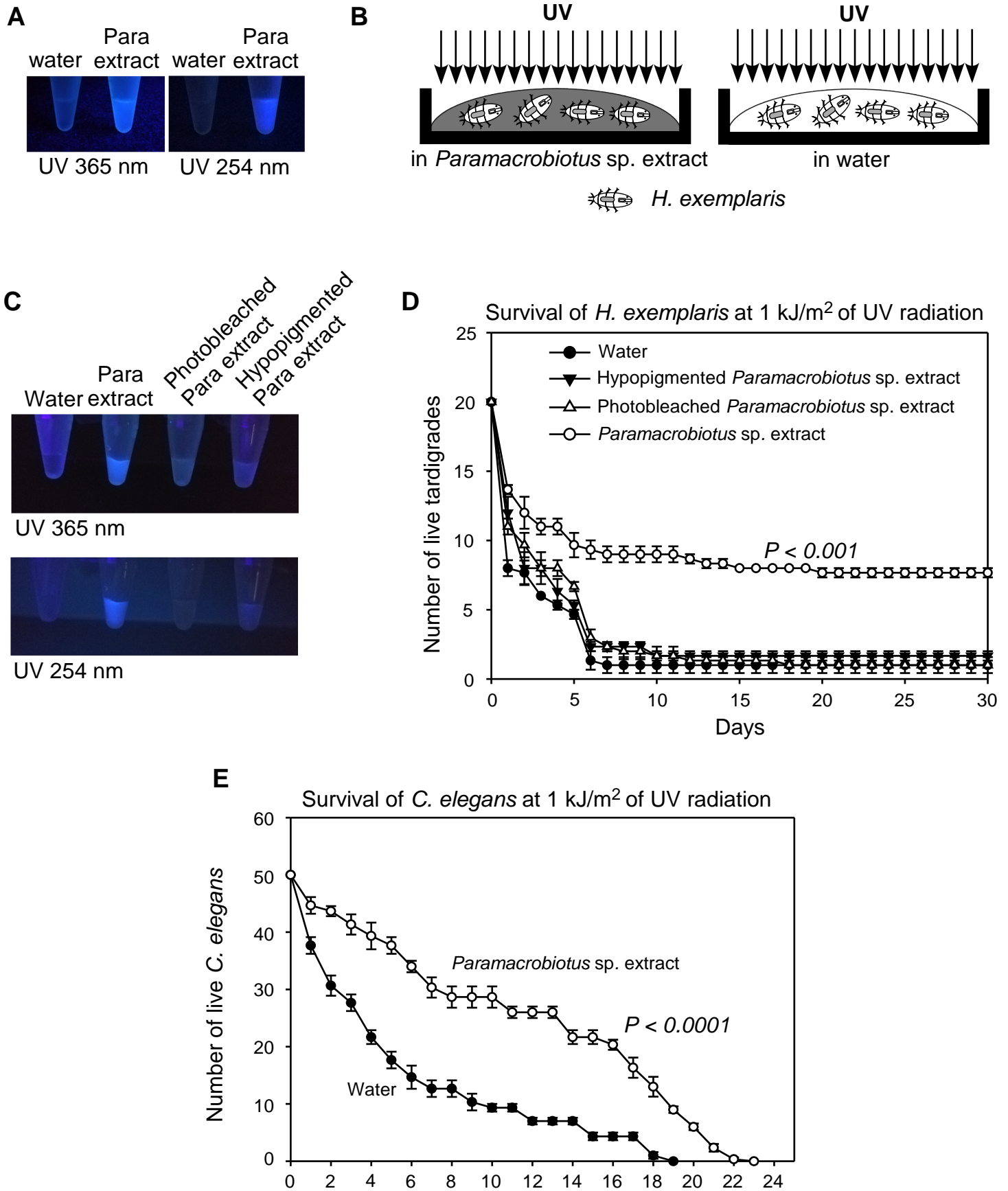


Figure 5



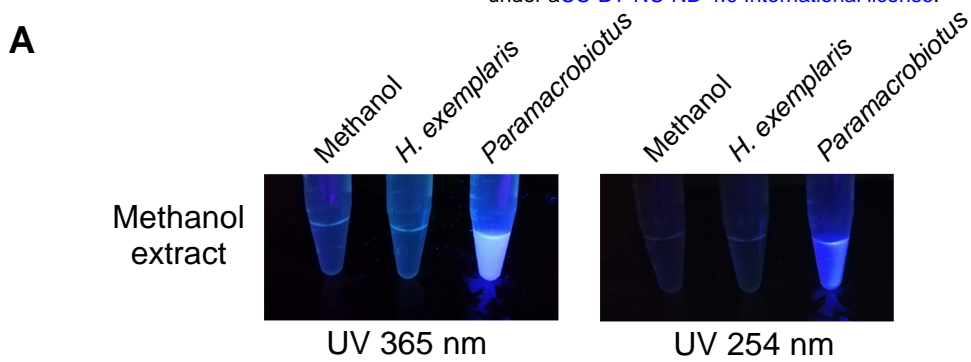


Figure 6

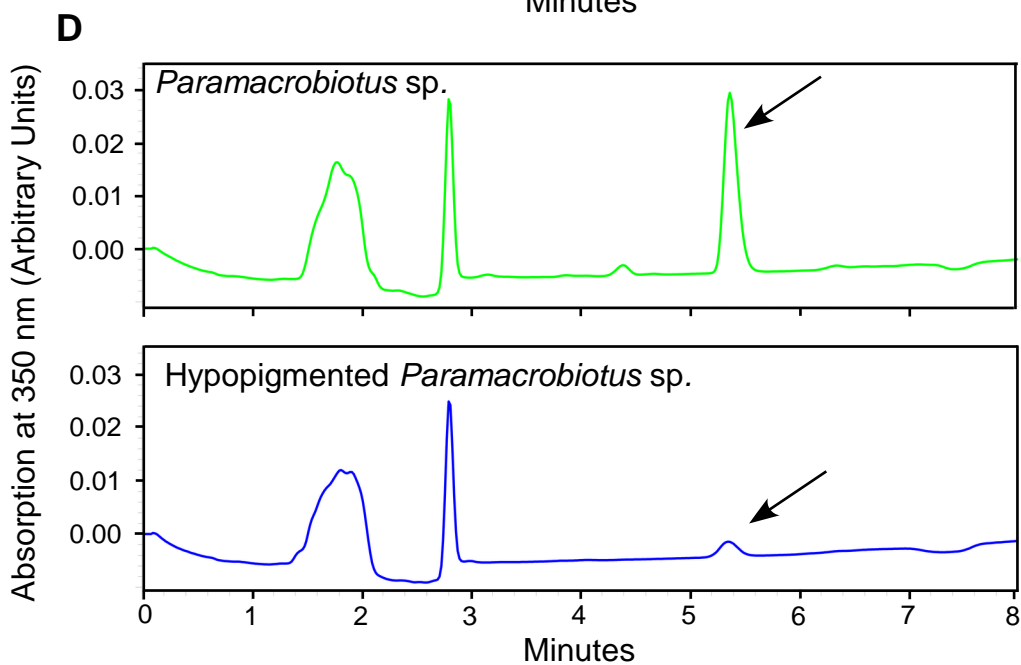
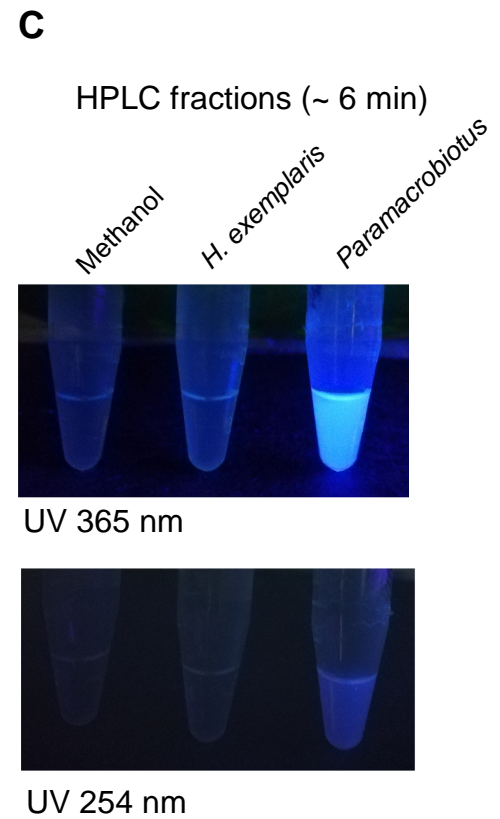
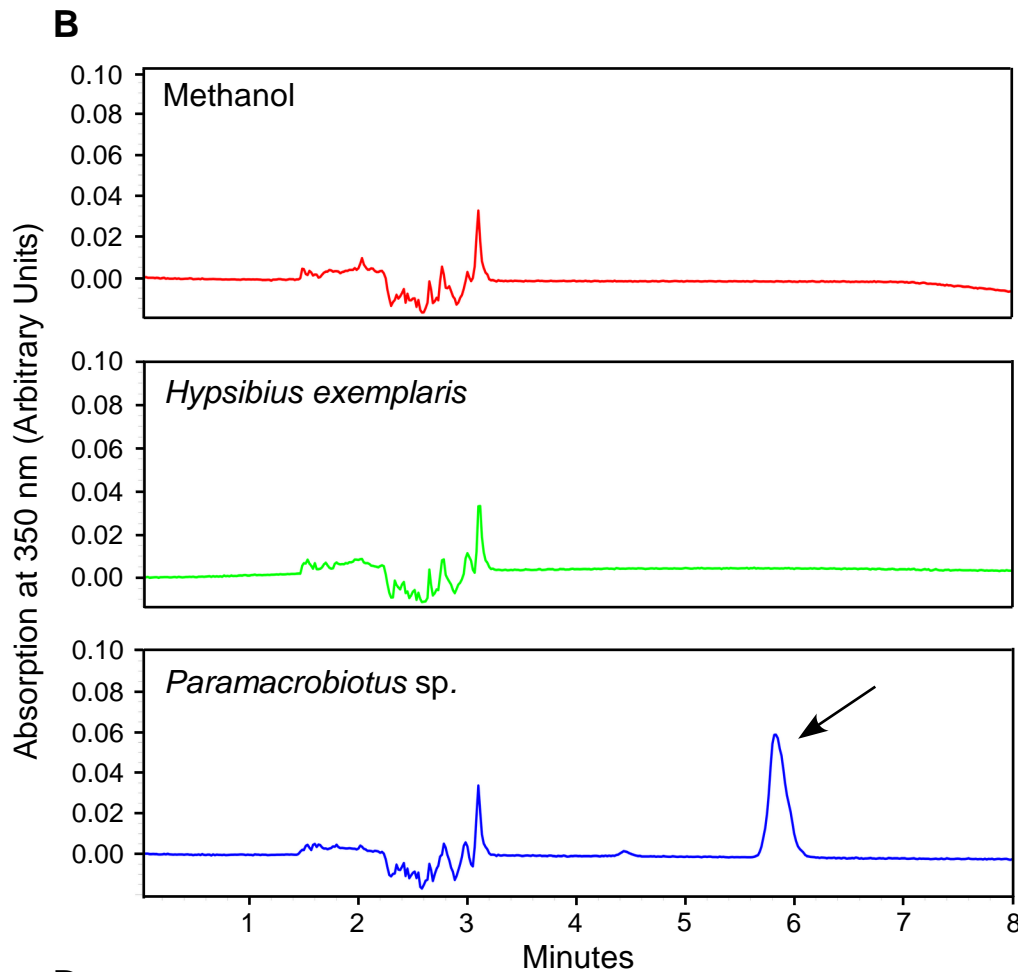


Figure 7

