

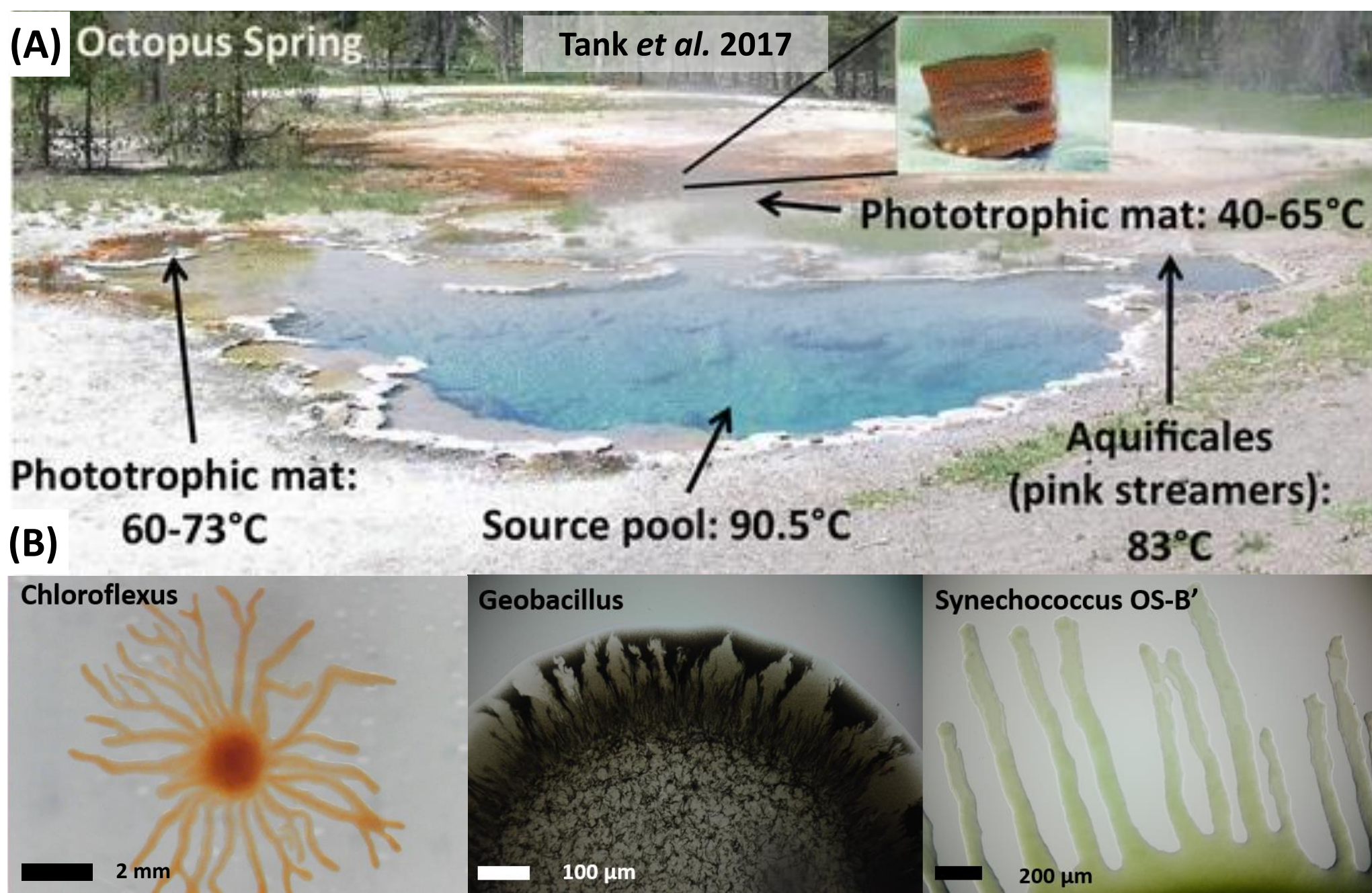
# Differential Phototactic Behavior of Closely Related Cyanobacterial Isolates from Yellowstone Hot Spring Biofilms

Freddy Bunbury, Carlos Rivas, Victoria Calatrava, Amanda N. Shelton, Andrey Malkovskiy, Arthur Grossman, Devaki Bhaya

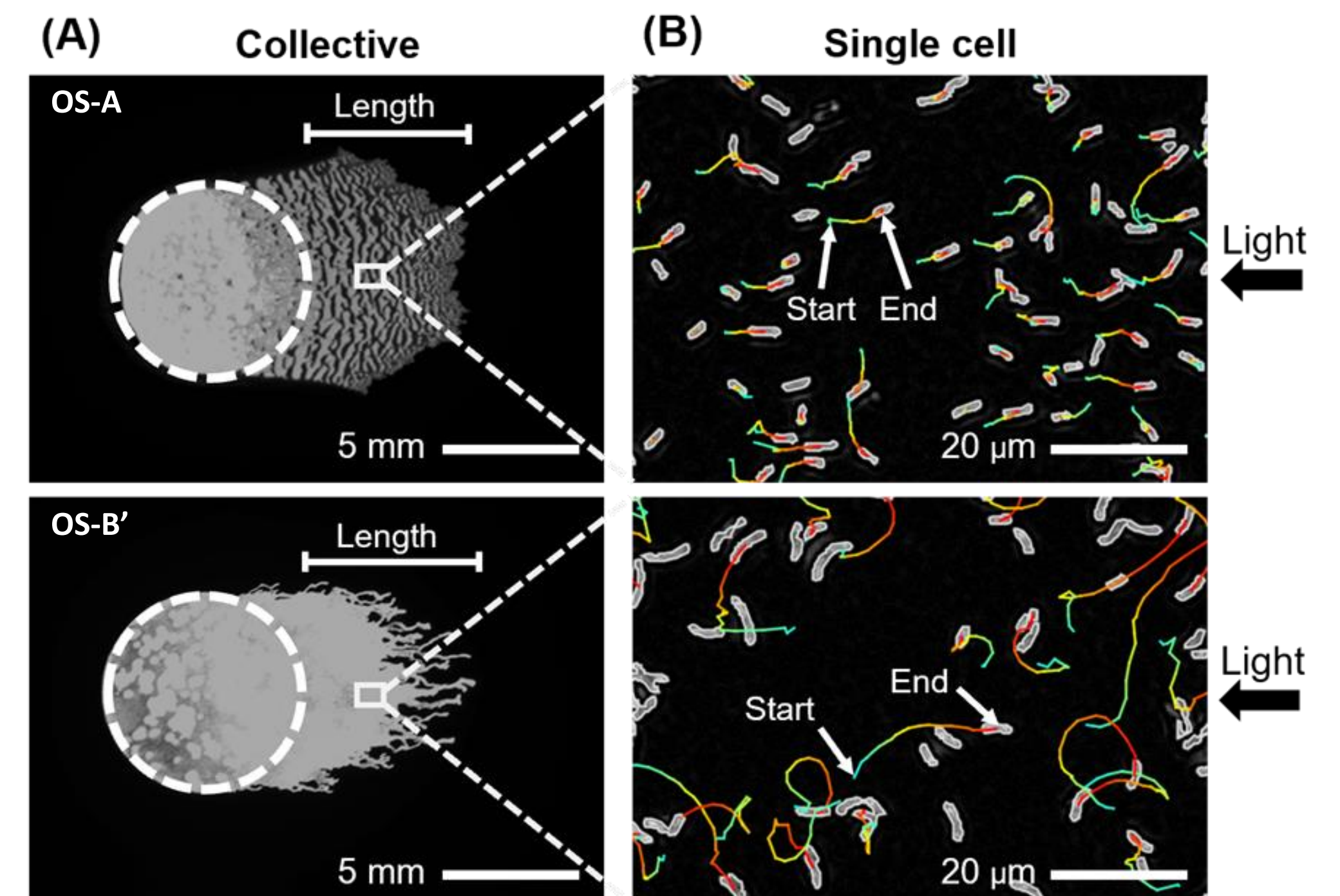
## Introduction

Cyanobacteria are keystone species in hot spring biofilms and show varied phototactic responses to the light environment.

- Yellowstone National park hot spring outflows are lined with dense and layered microbial biofilms.
- Synechococcus** is the keystone species at 50-70°C providing fixed carbon and nitrogen to the community.
- There are two dominant *Synechococcus* species: **OS-A** dominates at 60-65°C and **OS-B'** at 50-55°C.
- OS-A and OS-B' are also found at **different depths** in the biofilm and this changes over the diel cycle.
- We quantified the light dependent **motility** of OS-A and OS-B' at the **single cell** and **collective** level.
- We found differences between their **phototaxis** phenotypes and their **photoreceptor** sequences.
- Cyanobacteria have diverse photoreceptors and phototactic phenotypes suggesting **phototaxis is finely tuned** to the local light environment.

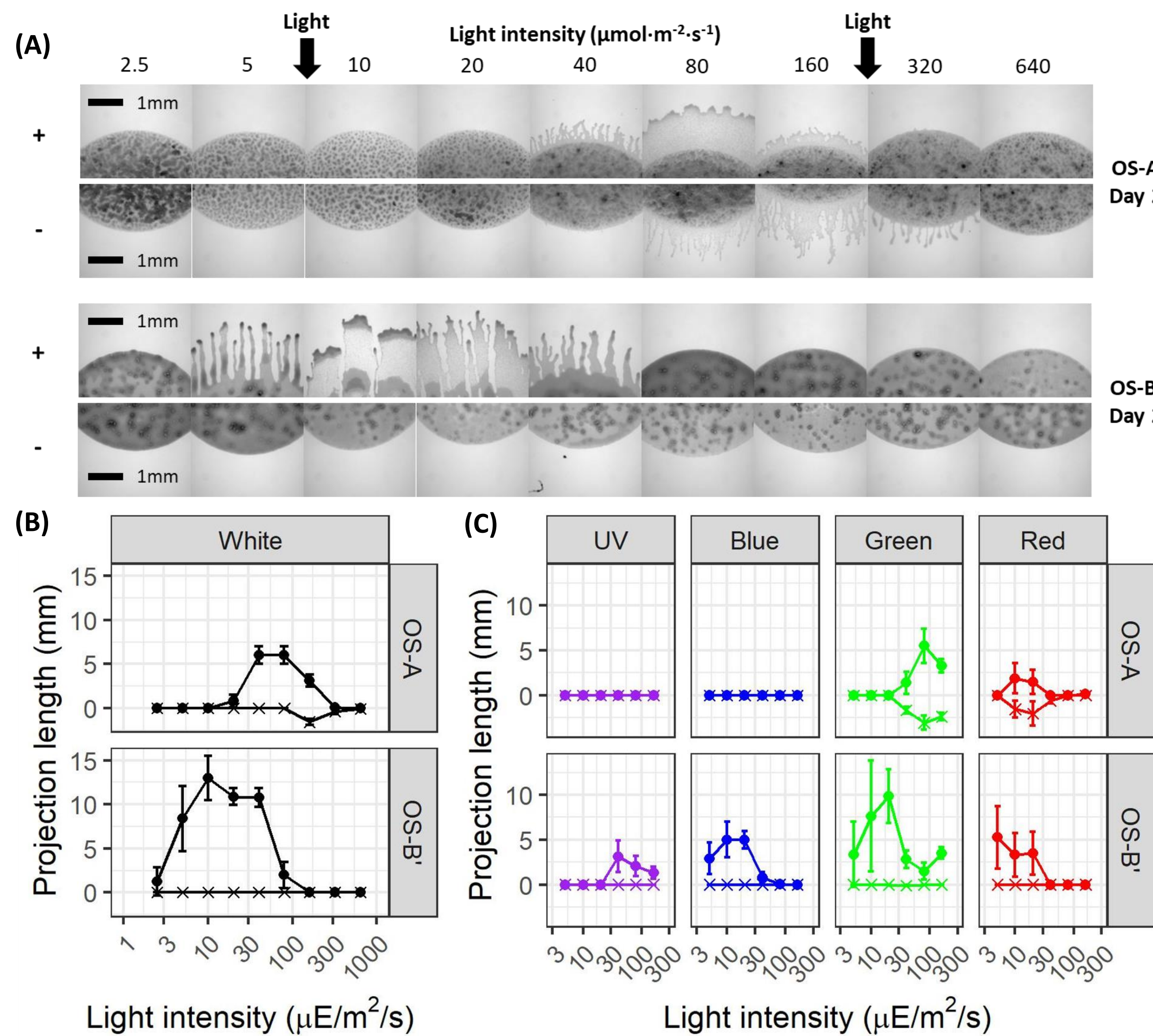


- Hot springs such as Octopus spring (shown above) in Yellowstone NP support thermophilic biofilm communities.
- Diverse phototrophic and heterotrophic bacteria can be isolated from the mat and many exhibit motility.

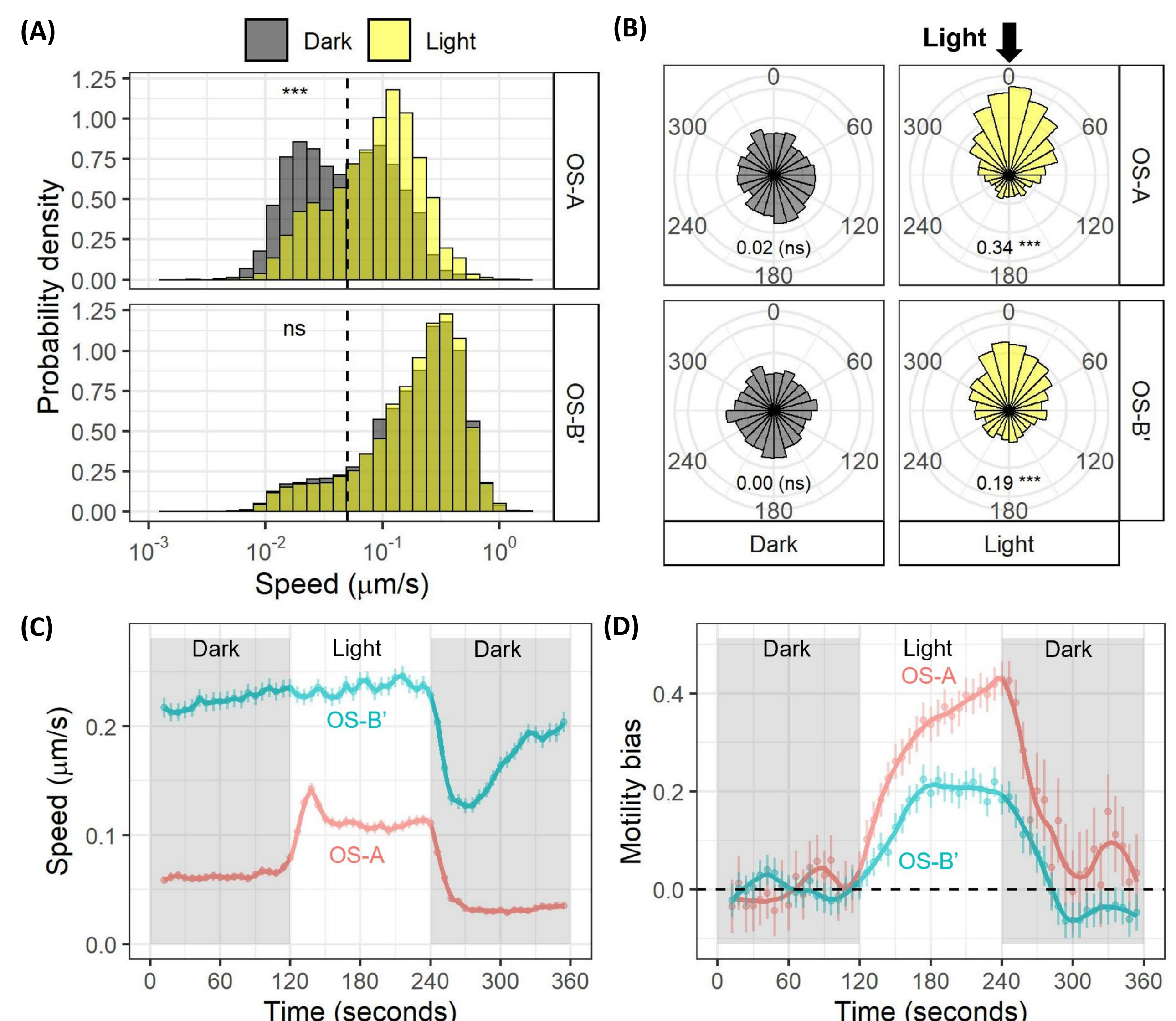


- OS-A and OS-B' mostly move collectively in groups but can also move as single cells.
- Particle tracking software allows us to quantify and analyze their dynamic responses to light.

## Quantifying phototaxis

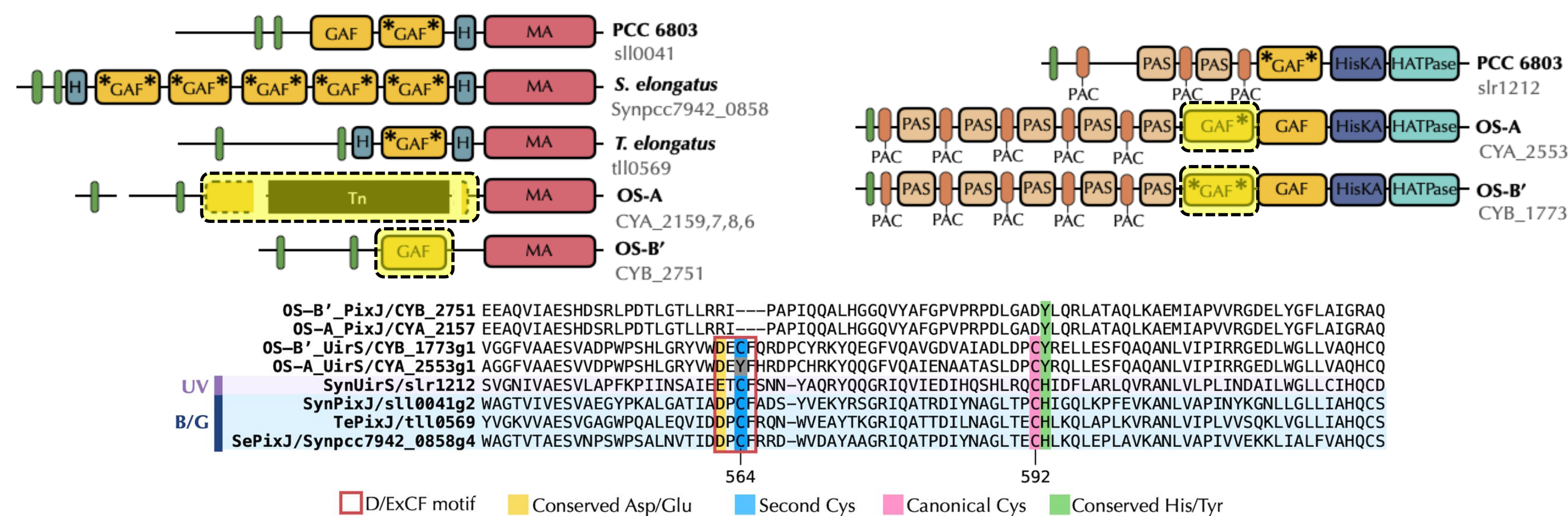


- OS-A performs more phototaxis at a higher light intensity than OS-B'.
- OS-A exhibits negative phototaxis but OS-B' does not.
- OS-B' responds to UV and blue light, but OS-A does not.



- OS-B' moves faster than OS-A.
- From dark to light, OS-A increases speed while OS-B' does not.
- OS-A has a larger phototactic bias than OS-B'.

## Phototaxis comparison with other cyanobacteria



- OS-A and OS-B' appear to lack a functional PixJ homolog.
- OS-B' has both bilin binding cysteines in the UirS homolog and OS-A only has one.

| Isolate/Species               | Environment     | Photo-kinesis | Positive phototaxis (bias value) | Negative phototaxis | Opt intensity ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | Phototaxis photoreceptors |
|-------------------------------|-----------------|---------------|----------------------------------|---------------------|---|---------------------------|
| <i>Synechococcus OS-A</i>     | Hot spring      | Yes           | Yes (0.3)                        | Yes                 | 40-80   | UirS                      |
| <i>Synechococcus OS-B'</i>    | Hot spring      | No            | Yes (0.2)                        | Yes (transient)     | 10-20   | UirS                      |
| <i>Synechococcus C1</i>       | Hot spring      | Yes           | Yes (nd)                         | Yes                 | nd  | nd                        |
| <i>Synechococcus C9</i>       | Hot spring      | Yes           | Yes (nd)                         | Yes                 | nd  | nd                        |
| <i>T. elongatus BP1</i>       | Hot spring      | No            | Yes (nd)                         | No                  | 25-100  | PixJ, PixD                |
| <i>T. vulcanus NIES-2134</i>  | Hot spring      | nd            | Yes (nd)                         | Yes                 | 70  | PixD                      |
| <i>S. elongatus UTEX 3055</i> | Stream          | nd            | Yes (0.35)                       | Yes                 | 20  | PixJ                      |
| <i>Synechocystis PCC 6803</i> | Freshwater lake | Yes           | Yes (0.5)                        | Yes                 | 1-10  | PixD, PixJ, UirS          |

- Phototactic cyanobacteria encode only some homologs of *Synechocystis* photoreceptors.
- Cyanobacteria show +ve and -ve phototaxis over a range of light intensities.

## Conclusions

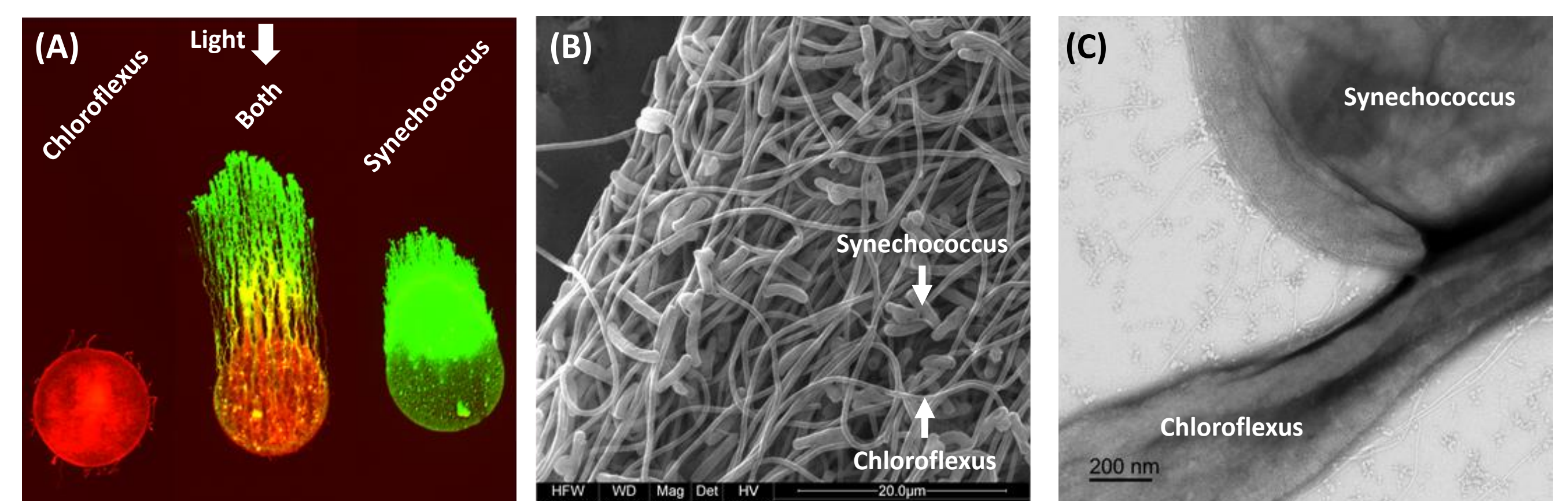
- Synechococcus* alters its motility in response to light quality, intensity and duration.
- OS-B' is faster, less phototactically biased, and more sensitive to low intensities than OS-A.
- Diverse cyanobacterial phototactic phenotypes and genotypes reflect different phototactic strategies.

## Future work

- Characterize the motility of other thermophilic bacteria from hot springs.
- Investigate the role of motility and physical interaction in building biofilm architecture.
- Determine the role of biofilm structure in facilitating species growth and nutrient exchange.

## Acknowledgements

- We thank our funding sources: NSF/BBSRC (1921429), Carnegie Institution for Science, and NASA (80NSSC19K0462).
- We thank the Yellowstone National Park Service and David Ward for sampling permit #YELL-5494.



- Synechococcus* and *Chloroflexus* cooperate to move further.
- Synechococcus* and *Chloroflexus* appear to form biofilms.
- Synechococcus* and *Chloroflexus* appear to attach to one another.