Title: VERVENet: The Viral Ecology Research and Virtual Exchange Network

Running Title: VERVENet: The Viral Ecology Research Network

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ABSTRACT

The advent of metagenomic methods to sequence DNA directly from an environment has revolutionized viral ecology, making it possible to "see" natural viral communities that could not be previously studied through culture. This technological leap has enabled further innovation in: extracting and sequencing limited viral DNA from communities, enriching and sequencing wild viruses through single-cell genomics, and creating new bioinformatics methods for large-scale comparative and functional metagenomics. Yet, the knowledge for specialized techniques in viral ecology remains in a subset of labs. We present a viral ecology community forum called VERVENet that strives to increase connectivity and knowledge dissemination in viral ecology research at all levels from undergraduates to accomplished viral ecologists. Our forum leverages and refines existing software from protocols.io to enhance a researcher's ability to: discuss and share protocols, connect with fellow community members, and learn about new and innovative research in the field. In delivering these valuable tools, VERVENet is a central resource to connect, collaborate, share and innovate for the viral ecology community. Moreover, these tools are broadly useful to any community or individual lab to promote scientific inquiry, reproduction of results, and dissemination and optimization of both molecular and bioinformatics protocols.

INTRODUCTION

The Internet has enabled for scientists online social interaction that previously happened only in physical meetings and conferences. Twitter, Facebook, and ResearchGate (Ellison et al. 2007; Kwak et al. 2010; Thelwall and Kousha 2015) provide valuable online forums where many researchers share knowledge. At the same time, academic publishing remains slow and particularly inefficient for communicating methodology. Protocols are often relegated to supplementary information, if shared at all. There is no good mechanism for easily discussing, troubleshooting, and improving published techniques.

This need is even more apparent in emerging fields such as aquatic viral ecology where lab, field, and bioinformatics methods are being actively developed in a subset of labs (Weinbauer et al. 2010). Further, given the experimental nature of these methods, the virus ecology community has expressed a need for fostering discussions about these protocols towards improved methodologies and to increase connectivity and collaboration among researchers. The challenge is to develop a method-centered collaborative platform that recapitulates the functionality of a scientific meeting - a digital community for connecting with fellow researchers to share and discover the state of the art.

Here, we present the Viral Ecology Research and Virtual Exchange Network (VERVENet), a collaboration between the University of Arizona and protocols.io, to deliver an online forum for the virus ecology community. This forum promotes scientific communication and collaboration to (*i*) fuel connectivity among viral ecology researchers for sharing data sets, knowledge, job postings, conference announcements through a common online forum called VERVENet, (ii) share protocols and their annotations and optimizations, and (*iii*) facilitate literature discovery through personalized recommendations to promote discussion on cutting edge viral ecology research. Through interconnecting these valuable resources, we have developed a "go-to" site for viral ecology research (2016k). Moreover, these tools are broadly useful to any community or individual lab for promoting scientific inquiry,

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reproduction of results, dissemination of protocols and re-use. Specifically, new forums can be created in a matter of minutes to enable connectivity among groups of any size, with tools described here.

RESULTS

The VERVENet Community Portal. The virus ecology field is in rapid development given new metagenomic techniques to sequence viral DNA directly from environmental samples (Brum and Sullivan 2015). These laboratory and analysis protocols, however, are highly specialized and generally only used in a few highly proficient labs because: (i) viral metagenomes (viromes) are difficult to produce due to low quantities of DNA and refined isolation and purification methods, (ii) the vast majority of viral sequences are unknown (usually >90% (Hurwitz and Sullivan 2013)) complicating bioinformatics analyses, and (iii) newly emerging comparative and functional metagenomic analyses exist but require on-going community refinement and development.

To allow for fast-paced innovation, the virus ecology community is interested in promoting discussions about lab, field and bioinformatic methods and increasing connectivity and collaboration among researchers. The challenge is to equip the community with a sustainable platform for these interactions -- a platform that capitalizes on online, mobile, and social network-based information exchange. The VERVENet Community portal at protocols.io addresses this need by providing: a broad forum for virus ecology, the ability to form subgroups by lab or area of interest, shared molecular and bioinformatics protocols, and a library equipped with a literature recommendation algorithm. The group forum and features are described in detail below, and are available to any community wishing to promote rapid community-driven development in their field.

Enabling Methods Discussion and Dissemination via protocols.io

Protocols.io was developed as a free service for industry and academic scientists to share or maintain private protocols for research (Teytelman and Stoliartchouk 2015). The driving force behind this software development is to provide a mechanism for scientists to share improvements and corrections to protocols, so that others are not continuously re-discovering knowledge that scientists have not had the time or wear-with-all to publish. Protocols.io provides a free, up-to-date, crowd-sourced protocol repository called protocols.io (http://www.protocols.io) for the life science community. This software is available as a web-based platform or smart phone App (ZappyLab 2016; 2016e) to enable mobile solutions for research and bench-work. Per best practices in mobile computing, these Apps offer extensive options and control of push notifications.

Protocols.io is open access and is both, free to read and free to publish. The revenue and sustainability model is based on the sale of data services to reagent vendors (most popular protocols, protocol improvements, and reagent-protocol links).

User profiles in protocols.io

Users can either view protocols anonymously, or interact directly by creating user profiles containing their name, email, website, affiliation, and research interests. Others can search and find that user based on name or keywords. Moreover, user profiles are attached to any material on protocols.io that the user posts publically. User profiles also contain a field for ORCID (Haak et al. 2012), so that researchers can

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tie their profile back to a common identifier and highlight their work in the field. Researchers can also include a biography that describes how they got into the field and what intrigues them.

Molecular and Bioinformatics Protocols

Often, detailed "tricks of the trade" associated with lab, field, and bioinformatics protocols are not well-described in publications, and at best are stashed in supplemental materials. Practical information associated with running these protocols under varied conditions cannot be curated, documented, or discussed among students, postdocs, technicians, and faculty working in virus ecology. Moreover, knowledge on when to use a particular version of a given protocol is not easily captured. Protocols.io provides a flexible mechanism wherein protocols can be documented in a stepswise fashion to easily pivot between molecular and bioinformatics methodologies, link to useful websites or code in Github (Dabbish et al. 2012), or reference manuals or original source materials for protocols.

The user entering the protocol may not necessarily be the author of the original method. However, by providing links to the primary work, users can attribute credit to the original author while at the same time adding their own updates to the method either while they enter it, or at a later time. Further, other users have the capability to add notes and warnings to existing protocols in protocols.io. This functionality includes a mechanism to email the protocol author for protocol troubleshooting. Corrections and updates made by the protocol authors and users automatically trigger notifications e-mailed to researchers who use that protocol Lastly, users can "fork" existing protocols for further refinement or alternate uses while still maintaining links back to the original for credit and reference. As such, the protocol is truly a living document for the community to reuse and continually refine.

For publication, authors have the option to enter detailed methods into protocols.io, issue a digital object identifier (DOI (Paskin 2008)), and link to the protocols.io record from the Methods section. This practice is now being encouraged in journal submissions, as in GigaScience data notes, and by funding agencies such as the Gordon and Betty Moore Foundation.

Protocol Collections

Because protocols are often times used in conjunction with other protocols, protocols.io has the capability to link protocols into user-defined workflows. This is particularly important for publications that may use a collection of varied protocols (field, lab, and bioinformatics) that are derived from many sources (protocols from the user or other users). In providing a collection of protocols associated with a publication, the authors enable their work to be replicated, easy to follow, and transparent to other members of the community in a way that can be referenced and cited. Thus, collections provide a mechanism for furthering open-science efforts.

Protocol collections can be used as a method to train new users in nuanced methodologies, or those specific to a focus area. This is particularly true for bioinformatics protocols that are often encoded in workflows including multiple programs and steps in an analysis for a given publication. Further, individual tools may have a collection of protocols that describe specific use-cases, example datasets, and varied options that they may wish to convey to their users. Refined collections of molecular protocols for specific scientific domains may also be best described in a collection, as in the case of the human skin double stranded DNA skin virome protocols (Hannigan et al. 2015; 2016j). Protocol collections also

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provide a mechanism to "learn by example" for early career scientists or those branching into a new area of scientific inquiry. In particular, detailed protocols associated with a toolkit or workshop, where multi-media options such as slides, video, or links to virtual machines with example datasets and code can be included (2016i; Caporaso et al. 2010).

Electronic Lab Notebook Capabilities

A core component of the protocols.io vision is a seamless integration of protocols with an electronic lab notebook. Experiments constantly require meticulous note-taking and cloning of the same protocol into one's notebook during each execution. The same is true for developing new methods in bioinformatics, wherein many iterations of code (or components in a workflow) need to be tested and refined given the analysis of resulting datasets. With the convenient snapshot capability on the web and mobile from each protocol, this functionality is key for protocol development and for facilitating crowdsourcing. Thus, scientists can use protocols.io within their day-to-day work, regardless of their desire to share or annotate protocols. Protocols can also be imported and edited to reflect the user's optimizations of any technique. After that, sharing the knowledge is just the tap of a button. protocols.io also includes reviews and ratings for reagents. Providing a"Yelp for reagents" is an important part of method discussion and knowledge sharing that can powerfully increase the reproducibility of research.

Groups and Sharing

Individual members can form groups with the group owner having the ability to choose the level of accessibility for fellow members. The groups are able to share literature recommendations, discussions, protocols, news, events, and job opportunities. Subgroups can form under the umbrella of a larger group with a common interest.This subgroup/supergroup relationship allows for a sharing of all of the group activities that have a common interest. In the case of VERVENet, this supergroup links the broader research of viral ecology with the subgroups of individual labs and more specific research interests such as plant viruses.

Literature Recommendation

Each of the groups comes equipped with a publication recommendation system called PubChase (2016h). This algorithm provides personalized publication recommendations based on a library from a user or group and is freely available to the broader community as a smart phone and web-based App. This algorithm is used to develop "libraries" for viral ecology user groups, that will continually recommend new publications based on growing reading lists from individual users that are part of the group. This functionality allows virologists using PubChase to make their reading lists public therefore helping new scientists joining the field in their topic area. The libraries from "sub-groups" also fuel the shared public reading list within the VERVENet group, therefore creating enhanced fluidity and cross-posted content between the groups.

Live Online Discussion Forum

Each of the groups in protocols.io contains a live on-line discussion. Discussions can be generated directly on the discussion tab, or are cross-posted from discussions on specific protocols, news, or literature. Each of the discussions can reference outside websites, manuals, or online resources. This discussion forum enables users to discuss tips and tricks for specific protocols, review reagents linked to particular protocols, and reference outside resources that were not included in the original protocol.

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Protocols.io also includes "journal-club" capabilities to enable on-line discussions of published research by researchers and authors. Other unique features in Protocols.io include: career advice forum including a panel of mentors (2016a) and a "*behind the article*" essay forum (2016b). These communication forums allow researchers to share their stories about how papers, protocols, or research efforts came about, that are both interesting to the community and informative for early career scientists.

Content and Adoption

The VERVENet group currently contains 292 live protocols, 116 news articles, and 49 job opportunities. There is an event calendar that is full of workshops and conferences specific to virus ecology through the fall of 2016. We have 114 members and 17 subgroups. The subgroups include: Plant Virus Ecology Network which originally formed in 2007 (Malmstrom et al. 2011), the Chlorovirus Group, ECOGEO (2016c), and 14 individual labs. The International Society for Viruses of Microorganisms has listed VERVE Net on their website (2016d) as a resource.

DISCUSSION

A primary goal of the VERVENet forum is to provide a robust web-application for sharing up to date protocols, literature, and community features (news, jobs, discussions) for virus ecologists. Fundamental to this goal is the ability for researchers to establish groups based on similar interests and share knowledge, without apriori knowledge of key members in a given field. Thus, we have designed an infrastructure that has multiple entry points for establishing relationships among users ranging from self-proclaimed groups or areas of interest, to options to join groups maintained by others in an area of interest to the user fueled by related protocols or reading lists. Moreover, news feeds about funding opportunities, job postings, or collaborative research opportunities can be fine-tuned according to interest. These connections will allow the forum to evolve naturally given rapidly developing trends and new protocols.

METHODS

Accessing protocols.io

A new protocol can be entered by clicking on your personal icon in the upper right hand corner and selecting '+ new protocol,' or by clicking on the protocols tab from within a group page and selecting '+ new protocol.' Initially you will be prompted to select whether you are entering a protocol, mixture, or collection with the drop-down arrow next to 'Protocol name' in the upper left next to the image box. You can then fill in the entry information under the 'description' tab, with the ability to share your protocol with any of your groups that you can access in the drop-down group button. The 'guidelines' tab allows for fully formatted text. The 'Steps' tab is where you will begin entering the protocol steps. A list of components you can add to your steps is located on the far right. When you hover over these components, you will see a start that you can select for items you will use frequently. Use the 'section' component to group together related steps of the protocol such as 'preparation,' 'DNA extraction,' and 'analysis' sections, etc. You may enter steps one by one by typing into the text box or by pasting steps from another file. The arrow to the left of the text box allows you to paste in large blocks of text that are numbered or separated by lines indicating individual steps. You may add a timer for each step by filling in the duration box. Protocols can be organized into sections/days using the 'section name.' For each step you can add annotations to make notes on specific steps. Additional steps are added by clicking on the '+' below the

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text box. Once the steps are complete, you will 'save changes' and 'close editor' below the protocol name. You are now able to run your protocol in a step-by-step format.

Once you have created a protocol, you have several options for sharing it. You will find those options in the toolbar directly above the steps tab. To make the protocol publicly viewable, you will click the 'publish' button. In this toolbar you will also find the ability to share the protocol with specific individuals or groups. You can reassign the protocol to another individual with a protocols.io account by hovering over the wheel and clicking 'reassign.' For ongoing development and changes to adding and using protocols, see tutorials in protocols.io (2016g).

Developing groups in protocols.io

To create a group, you must have an account and be logged in. You will then click on your personal icon in the upper right hand corner and select "+ new group." You will be prompted to enter a group name, image, description of your group, research interests, external website address, physical location of your group, and an affiliation. You will also decide if your group is open to anyone, by invitation only, or open to membership requests. In addition, you can choose if your group is visible others or private. Once you have saved the initial information, you can always go back and edit your group by hovering over the wheel that is located directly below your group image in the upper left hand corner of your group page. You are able to invite members into your group and control the privileges of your members by hovering over this same wheel and choosing 'manage members.'

As the owner of a group, you are able to invite other groups to become subgroups. There is an invitation below the description of a group on the 'about' page. You will be prompted to select your supergroup, then an invitation is sent to the subgroup owner.

Adding content to groups

You can add news items by clicking on the 'News' tab, then selecting "+ add news" to the left of the search bar. You will then be prompted to add an image, title, and formatted text. Events such as workshops or conferences can be added by going to the 'Events' tab and selecting "+ new event". You will be prompted to add a title, formatted description, external link, location, start and end dates, and organizer. Once you have saved the event, it will appear in both a calendar and list view. Job opportunities can be added by clicking on the tab and selecting "+ New job." You can then enter a title, formatted description, organization, qualifications, location, pay, external url, and contact email address. You can choose to share all of this content with super/sub groups on the entry page.

You can create a library of journal articles under the 'Articles' tab. You can search through PubMed to initially seed your library in the search bar to the right. Your search results can be added to your library by clicking the "+" to the right of the title. Once you have added approximately 50 articles to your library, you will begin getting literature recommendations. Both your library and your recommendations can be searched by clicking the arrow to the left of the search bar. You can also choose to share your library and recommendations with the group by checking 'Share' above the list of articles in your library.

Platform Infrastructure and Interoperability

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Computers, tablets, and smart phones are becoming fundamental tools for scientists today. Furthermore, social networking and shared cyberinfrastructures are offering powerful new mechanisms to connect communities and science from across the world. protocols.io leverages these powerful new tools and software capabilities to provide an online forum for viral ecology research to connect and share knowledge and resources. Specifically, all components of protocols.io and the VERVENet forum are mobile-friendly and interoperable for use on diverse devices in the lab, on the desktop, or on the go.

DATA ACCESS

Protocols.io and the VERVENet commuity forum are committed to open access and interoperability. To that end, the VERVENet on-line forum is built on the protocols.io platform where other forums and groups can be easily integrated into the same infrasturcture. To enable stable digital archiving, all content will be mirrored in partnership with the Center for Open Science and CLOCKSS.

Application Programming Interface (API) for protocols.io and Pubchase

To promote open-science efforts an API was developed for protocols.io and PubChase for advanced data mining. Details on the API and use are documents on the protocols.io website (2016f).

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1. The VERVENet group in protocols.io. Groups in protocols.io display information about the group objectives, members, subgroups, the group library and literature recommendations, group discussions, news, jobs, and events. Groups have the capacity to control access, from making groups and content public and allowing anyone to join, to restricted content and invitation only membership. VERVENet is an example of a public forum for virus ecology.

Figure 2. Entering a protocol in protocols.io. Protocols are entered by providing a broad description, information about authors, any prior materials or background required, and detailed step-by-step methods to implement the protocol. Protocols can remain private to an individual or group, or released to the public.

Figure 3. Library and literature recommendation in protocols.io. The VERVENet forum maintains a group library that is comprised of literature from members, subgroups, or articles directed added to the VERVENet library by members. Literature recommendations are made using the Pubchase algorithm to add relevant new literature on a weekly basis for the group.

TABLES

None.

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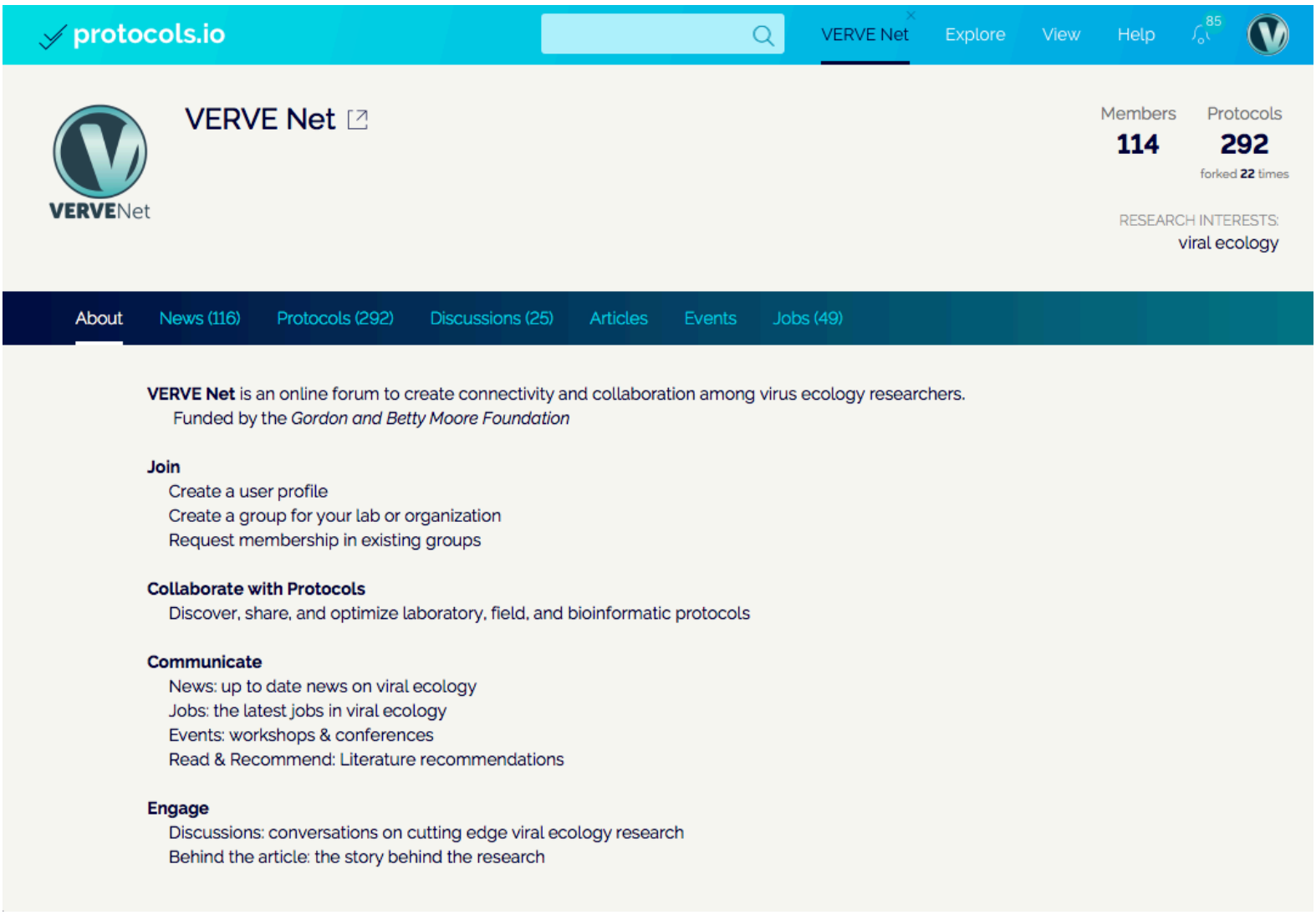

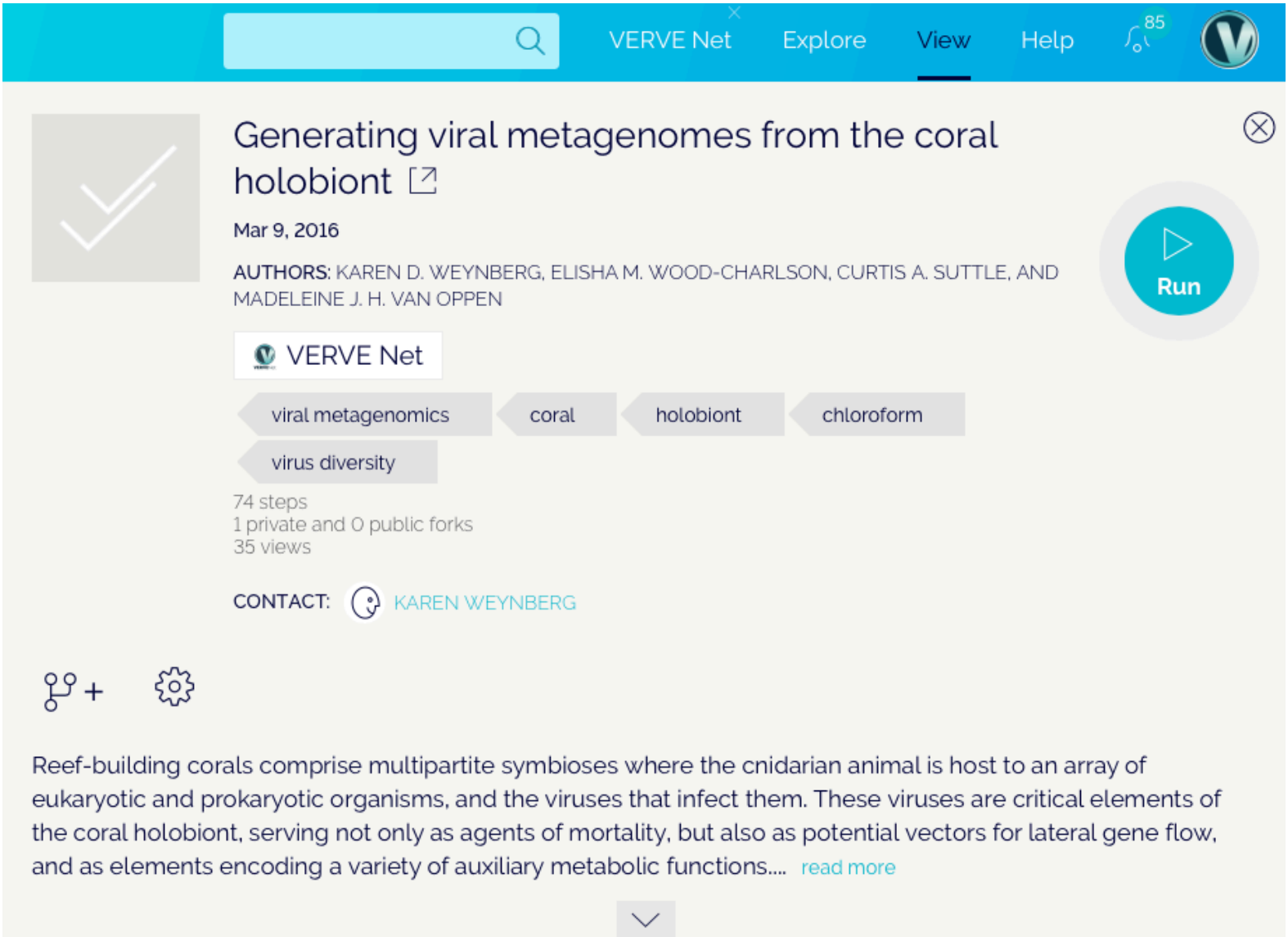

VERVE Net

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Faustovirus-Like Asfarvirus in Hematophagous Biting Midges and Their Vertebrate Hosts

TEMMAM S, MONTEIL-BOUCHARD S, SAMBOU M, AUBADIE-LADRIX M, AZZA S, DECLOQUEMENT P, KHALIL JY, BAUDOIN JP, JARDOT P, ROBERT C, LA SCOLA B, MEDIANNIKOV OY, RAOULT D, DESNUES C

Jan 6, 2016 **Frontiers in microbiology**

Faustovirus, a new Asfarviridae-related giant virus, was recently isolated in Vermamoeba vermiformis, a protist found in sewage water in various geographical locations and occasionally reported in human eye infection cases. As part of a global metagenomic analysis of viral communities existing in biting midges, we report here for the first time the identification and isolation of a Faustovirus-like virus in hematophagous arthropods and its detection in their animal hosts. The DNA virome analysis of three pools of Culicoides sp., engorged... read more

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Discovery, Prevalence, and Persistence of Novel Circular Single-Stranded DNA Viruses in the Ctenophores Mnemiopsis leidyi and Beroe ovata

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Jan 6, 2016 **Frontiers in microbiology**

Gelatinous zooplankton, such as ctenophores and jellyfish, are important components of marine and brackish ecosystems and play critical roles in aquatic biogeochemistry. As voracious predators of plankton, ctenophores have key positions in aquatic food webs and are often successful invaders when introduced to new areas. Gelatinous zooplankton have strong impacts on ecosystem services, particularly in coastal environments. However, little is known about the factors responsible for regulating population dynamics of gelatinous organisms,... read more

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