

1. To deploy the speCOIdent application on your computer, it is necessary to install specific packages which are listed at the start of the app.R file in this repository: <https://github.com/MutinAndrei/Shiny-web-application-for-analyzing-genotyping-results>.
2. Along with the necessary packages, you will require a FASTA file (your_file.fa) and a CSV file containing latitude and longitude data for your sequences. An example of such files can be found at <https://github.com/MutinAndrei/Shiny-web-application-for-analyzing-genotyping-results>.
3. To begin, install BLAST+ (<https://www.ncbi.nlm.nih.gov/books/NBK569861/>) on your computer and create a BLAST database from your .fasta file. Detailed instructions on this process can be found here: <https://www.ncbi.nlm.nih.gov/books/NBK569841/>.
4. Upon successful creation of the database, there will be six files with the extensions .nhr .nin .nog .nsd .nsi .nsq. An exemplary database can be found at <https://github.com/MutinAndrei/Shiny-web-application-for-analyzing-genotyping-results>.
5. After completing step 4, you should have the application script downloaded from <https://github.com/MutinAndrei/Shiny-web-application-for-analyzing-genotyping-results> and a table containing

Table displaying latitude and longitude coordinates to be created using the example provided on the table from <https://github.com/MutinAndrei/Shiny-web-application-for-analyzing-genotyping-results>.

Files with extensions .nhr .nin .nog .nsd .nsi .nsq (example: <https://github.com/MutinAndrei/Shiny-web-application-for-analyzing-genotyping-results>)

Fasta file your_file.fa.

6. Are all the files in place? You can move on!
7. Open the script downloaded from the GitHub repository at <https://github.com/MutinAndrei/Shiny-web-application-for-analyzing-genotyping-results>. Modify lines 180 and 181 as follows: change "custom_db <-

```
read.fasta("database_specoident999.fa")"          to          "custom_db          <-
read.fasta("your_file.fa")" (row 180) and change "custom_db_path  <-
c("database_specoident99.blastdb")"          to          "custom_db_path  <-
c("your_database_data.blastdb")" (row 181).
```

8. Change line 214 (*coord <- read.csv("table_lang_and_latit.csv")* change to *coord <- read.csv("your_table_with_coordinates.csv")*)

9. It is necessary to modify the identification pattern for your species from lines 286 to 492 (287, 294, 302, 309, 316, 323, 337, 344, 351, 358, 365, 372, 379, 386, 393, 400, 414, 421, 428, 435, 442, 449, 456, 463, 470, 477, 484). To prevent complications, if you have more or fewer species, it is advisable to use abbreviations within the ifelse.

Example 1.

Two biological species are present, equivalent to two patterns. However, the script includes an extra pattern. To adjust for this, modify the search patterns and remove one branch of the condition, resulting in two patterns as intended.

Please note that the search pattern pertains specifically to one species in the fasta file, with all sequences of that species including this identifier in their name.

```
Example: >E_B1_Eulimnogammarus_verrucosus(your_pattern1)_18S
GTGATTATTCGGTCTGAGCTAAGAACACCTGGTAATCTAATCGGAG
ATGACCAGTTATATAATGTTATAGTAACAGCTCACG
>E_B2_Eulimnogammarus_verrucosus(your_pattern2)_18S
GTGATTATTCGGTCTGAGCTAAGAACACCTGGTAATCTAATCGGAG
ATGACCAGTTATATAATGTTATAGTAACAGCTCACG
>E_2K2_Eulimnogammarus_verrucosus(your_pattern1)_18S
GTGATTATTCGGTCTGAGCTAAGAACACCTGGTAATCTAAACCTGT
T
>E_2K4_Eulimnogammarus_verrucosus(your_pattern2)_18S
GTGATTATTCGGTCTGAGCTAAGAACACCTGGTAATCTAATCGGAG
ATGACCAGTTATATAATGTTATAGTAACAGCTCACG
```

(your_Pattern1)_COI combines sequences 1 and 3.

This is necessary to adequately represent the range map.

You need to change .west._18S to (your_Pattern1)_18S (dots in .west. denote brackets), .eastern._18S to your_Pattern2_18S, etc. (If you have more species)

It was

```
observeEvent(input$blast, {
```

```

    if (str_detect(parsedresults()[1, "Species"], ".west._18S") == TRUE) {
      showModal(modalDialog(
        title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
        HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (eastern)"),
        easyClose = TRUE,
        footer = NULL, size = "xl"
      ))
    } else if (str_detect(parsedresults()[1, "Species"], ".eastern._18S") == TRUE) {
      showModal(modalDialog(
        title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
        HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (west)"),
        easyClose = TRUE,
        footer = NULL,
        size = "xl"
      ))
    } else if (str_detect(parsedresults()[1, "Species"], ".southern._18S") == TRUE) {
      showModal(modalDialog(
        title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
        HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (southern)"),
        easyClose = TRUE,
        footer = NULL, size = "xl"
      ))
    } else {
      showModal(modalDialog(
        title = "No matches found!",
        HTML("If your % ID is greater than 95 try lowering it"),
        easyClose = TRUE,
        footer = NULL, size = "m"
      ))
    }
  })
}
})

```

It became

```

observeEvent(input$blast, {
  if (str_detect(parsedresults()[1, "Species"], ".your_pattern1._18S") == TRUE) {
    showModal(modalDialog(
      title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
      HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (eastern)"),
      easyClose = TRUE,
      footer = NULL, size = "xl"
    ))
  } else if (str_detect(parsedresults()[1, "Species"], ".west._18S") == TRUE) {
    showModal(modalDialog(
      title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
      HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (west)"),
      easyClose = TRUE,
      footer = NULL,
      size = "xl"
    ))
  } else if (str_detect(parsedresults()[1, "Species"], ".your_pattern2._18S") == TRUE) {

```

```

        showModal(modalDialog(
            title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
            HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (southern)"),
            easyClose = TRUE,
            footer = NULL, size = "xl"
        ))
    } else {
        showModal(modalDialog(
            title = "No matches found!",
            HTML("If your % ID is greater than 95 try lowering it"),
            easyClose = TRUE,
            footer = NULL, size = "m"
        ))
    }
})

```

10. Great job on modifying the search pattern, yet there is an extraneous condition branch. To fix this, remove the branch within the condition in order to maintain its validity.

It was

```

observeEvent(input$blast, {
  if (str_detect(parsedresults()[1, "Species"], ".your_pattern1._18S") == TRUE) {
    showModal(modalDialog(
      title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
      HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (eastern)"),
      easyClose = TRUE,
      footer = NULL, size = "xl"
    ))
  } else if (str_detect(parsedresults()[1, "Species"], ".your_pattern2._18S") == TRUE) {
    showModal(modalDialog(
      title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
      HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (southern)"),
      easyClose = TRUE,
      footer = NULL, size = "xl"
    ))
  } else {
    showModal(modalDialog(
      title = "No matches found!",
      HTML("If your % ID is greater than 95 try lowering it"),
      easyClose = TRUE,
      footer = NULL, size = "m"
    ))
  }
})

```

It became

```

observeEvent(input$blast, {
  if (str_detect(parsedresults()[1, "Species"], ".your_pattern1._18S") == TRUE) {

```

```

showModal(modalDialog(
  title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
  HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (eastern)"),
  easyClose = TRUE,
  footer = NULL, size = "xl"
))
} else if (str_detect(parsedresults()[1, "Species"], ".ваш_Паттерн2._18S") == TRUE) {
showModal(modalDialog(
  title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
  HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (southern)"),
  easyClose = TRUE,
  footer = NULL, size = "xl"
))
} else {
showModal(modalDialog(
  title = "No matches found!",
  HTML("If your % ID is greater than 95 try lowering it"),
  easyClose = TRUE,
  footer = NULL, size = "m"
))
}
})

```

11. Changing the popup message.

The message can be found within the quotation marks under the HTML("MESSAGE"). Please modify the highlighted portion.

It was

```

observeEvent(input$blast, {
  if (str_detect(parsedresults()[1, "Species"], ".your_pattern1._18S") == TRUE) {
    showModal(modalDialog(
      title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
      HTML("This sequence, with more than 95% identity, belongs to the species:
<b><i>Eulimnogammarus verrucosus</i></b> (eastern)"),
      easyClose = TRUE,
      footer = NULL, size = "xl"
    ))
  } else if (str_detect(parsedresults()[1, "Species"], ".your_pattern2._18S") == TRUE) {
    showModal(modalDialog(
      title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
      HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (southern)"),
      easyClose = TRUE,
      footer = NULL, size = "xl"
    ))
  } else {
    showModal(modalDialog(
      title = "No matches found!",
      HTML("If your % ID is greater than 95 try lowering it"),

```

```

        easyClose = TRUE,
        footer = NULL, size = "m"
    ))
}
})

```

It became

```

observeEvent(input$blast, {
  if (str_detect(parsedresults()[1, "Species"], ".your_pattern1._18S") == TRUE) {
    showModal(modalDialog(
      title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
      HTML("Your species that you define"),
      easyClose = TRUE,
      footer = NULL, size = "xl"
    ))
  } else if (str_detect(parsedresults()[1, "Species"], ".your_pattern2._18S") == TRUE) {
    showModal(modalDialog(
      title = "This sequence is the 18S (small subunit of ribosomal DNA) gene",
      HTML("This sequence, with more than 95% identity, belongs to the species: <b><i>Eulimnogammarus
verrucosus</i></b> (southern)"),
      easyClose = TRUE,
      footer = NULL, size = "xl"
    ))
  } else {
    showModal(modalDialog(
      title = "No matches found!",
      HTML("If your % ID is greater than 95 try lowering it"),
      easyClose = TRUE,
      footer = NULL, size = "m"
    ))
  }
})

```

12. DONE! Once you have completed modifying all of the conditional branches, you can execute the application. Either click the 'runapp' button or select all and run the script.

If you have any questions, please contact andreimutin97@gmail.com